2016

Factors affecting mercury concentrations in Iowa fishes

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by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Fisheries Biology

Program of Study Committee:
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Iowa State University

Ames, Iowa

2016

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DEDICATION

I dedicate this thesis to my family, friends, and to the memory of my grandfather, Harvey Mills, for the support they have given me throughout this project.

ACKNOWLEDGEMENTS

This project is funded by the Iowa Department of Natural Resources through contract 14CRDFBGSCHO-0002 and Iowa State University as part of an ongoing evaluation of mercury contamination in Iowa fishes. Special thanks to Iowa Department of Natural Resources Fisheries Biologist, Darcy Cashatt, for her advice, patience, and dedication to the success of this project. I would also like to thank other fisheries personnel throughout Iowa that have assisted with fish collection and provided fishes for this project. Finally, I would like to thank Adam Martin-Schwarze with the Statistics Department at Iowa State University for helping with the statistical analyses used in this study. This study was performed under the auspices of Iowa State University Institutional Animal Care and Use Committee (IACUC) protocol permit 4-14-7780-I, and animals were collected under state permit SC1037. Use of trade, product, or firm names is descriptive and does not imply endorsement by the U.S. Government.

ABSTRACT

Mercury contamination in aquatic ecosystems is a global concern due to the health risks of consuming contaminated aquatic organisms, particularly fishes. Mercury concentrations in fishes are highly variable and influenced by a range of biotic and abiotic variables. Currently, factors influencing mercury accumulation in Iowa fishes are not well understood. The Iowa Department of Natural Resources (IDNR) has issued fish consumption advisories for various lakes and river reaches throughout the state. However, relatively few systems, species, and individuals are sampled each year and little is known regarding factors affecting mercury concentrations in Iowa fishes. An understanding of factors regulating mercury concentrations in Iowa fishes would improve mercury monitoring programs and consumption guidelines. The objectives of this study were to (I) evaluate seasonal variation in mercury concentrations in largemouth bass (*Micropterus salmoides*) from two reservoirs to assess the need for temporally standardized mercury sampling and (II) evaluate the influence of a suite of biotic and abiotic factors on fish mercury concentrations in both river and lake systems. Largemouth bass were intensively sampled from Red Haw and Twelve Mile lakes to evaluate temporal variation in largemouth bass mercury concentrations. Bluegill (*Lepomis macrochirus*, n = 275), white and black crappie (*Pomoxis annularis*, n = 112; *P. nigromaculatus*, n = 203), largemouth bass (n = 502), walleye (*Sander vitreus*, n = 248), muskellunge (*Esox masquinongy*, n = 30), and northern pike (*E. lucius*, n = 45) were collected between April and October, 2013-2015, from natural lakes (n = 6), shallow natural lakes (n = 2), constructed lakes (n = 18), and reservoirs (n = 4) throughout Iowa. Additionally, channel catfish (*Ictalurus punctatus*, n = 205), flathead catfish (*Pylodictis olivaris*, n = 123), northern pike (*Esox lucius*, n = 60), smallmouth bass (*Micropterus dolomieu*, n = 176), and walleye (*Sander vitreus*, n = 176) were collected between March and

October, 2014-2015, from ten Iowa interior rivers and tested for mercury contamination. Fish were collected from an upstream and a downstream location on six of the rivers to test for intra-river differences in fish mercury concentrations. Various land use, water chemistry, and fish characteristics were gathered and used to explain differences in mercury concentrations within and across lake and river systems. Largemouth bass mercury concentrations varied across months in Red Haw Lake, with the highest concentrations observed during July, and the lowest concentrations observed during October. In contrast, largemouth bass mercury concentrations were similar across months in Twelve Mile Lake. Fish mercury concentrations in Iowa lakes are generally low, with mercury concentrations <0.30 mg/kg for ~90% of fishes collected and mercury concentrations below detectable levels (<0.05 mg/kg) for ~40% of fishes. Multiple linear regression models, sorted by AIC_c, were used to evaluate factors related to fish mercury concentrations in lakes and rivers. Detected mercury concentrations were highest in muskellunge, northern pike, walleye and largemouth bass, lowest in black and white crappie and bluegill, and positively related to fish length and age. Although not significantly different across all species, females generally had higher mercury concentrations than males. Additionally, pH, lake mean depth, watershed area to lake area ratio, and percent of watershed as forested land, grasslands and open water were positively related to fish mercury concentrations, whereas lake area and percent of watershed as agriculture and developed land were negatively related to mercury concentrations. Finally, detected mercury concentrations were on average 28% higher in shallow natural lakes compared to other lake types. Combined, these factors explained 74% of the variation in detectable fish mercury concentrations. Fish mercury concentrations in Iowa rivers were also generally low (mean = 0.17 mg/kg, N = 740). Mercury concentrations were highest in flathead catfish, northern pike, smallmouth bass, and walleye but

lowest in channel catfish. Fish mercury concentrations were positively related to length, age, trophic position and $\delta^{13}C$ signatures. Human Threat Index and percent of watershed as open water were negatively related to fish mercury concentrations, whereas percent of watershed as forested land was positively related to fish mercury concentrations. Additionally, phosphorous (mg/L), nitrogen-ammonia (mg/L), and sulfate (mg/L) were weakly negatively related to mercury concentrations, whereas water hardness (as $CaCO_3$, mg/L) was weakly positively related to fish mercury concentrations. Additionally, fishes collected from the Paleozoic Plateau ecoregion had the highest mercury concentrations compared to those collected from other ecoregions across Iowa. Together, these factors explained 70% of the variation in river fish mercury concentrations. Results of this study suggest less impacted watersheds, particularly watersheds with less agricultural impacts, tend to have higher fish mercury concentrations compared to watersheds that have a high proportion of agriculture. This study provides a comprehensive analysis of abiotic and biotic factors influencing fish mercury concentrations in Iowa and may have implications for refining consumption advisories.

CHAPTER 1

GENERAL INTRODUCTION

Mercury is a harmful neurotoxin of global concern due to its relatively recent upsurge in the environment. Mercury availability in aquatic systems depends on both natural and anthropogenic inputs (Pirrone et al. 2010). Natural sources collectively contribute 69% of annual atmospheric mercury emissions and include gaseous emissions from volcanoes, geothermal sources, and mercury enriched topsoil (Mason 2009; Pirrone et al. 2010). Although mercury sources vary regionally across the landscape, direct geological mercury inputs are typically negligible compared to atmospheric inputs (Swain et al. 1992). Natural re-emission processes that transfer mercury from geological sources to the atmosphere include volatilization from ocean and inland waters and biomass burning (Mason 2009; Pirrone et al. 2010).

Although mercury is a naturally present element, anthropogenic mercury inputs have largely increased in the 19th and 20th centuries due to industrial processes (Driscoll et al. 2007). Significant anthropogenic sources of mercury include fossil-fuel and coal burning power plants, gold mining, concrete production, and non-ferrous metal mining (Driscoll et al. 2007; Pirrone et al. 2009, 2010). Currently, coal-fired power plants are the largest point source, contributing 35% of the total anthropogenic mercury emissions (Pirrone et al. 2010). The United States has reduced mercury emissions by almost 60% since 1990 to reduce mercury contamination (Wentz et al. 2014). However, mercury can be transported long distances in the atmosphere and worldwide anthropogenic mercury emissions are still an important issue. For example, China's mercury emissions are more than 4-times higher than that of the U.S. (Feng et al. 2009; Streets et al. 2009).

Various chemical forms of mercury are emitted into the atmosphere from both natural and anthropocentric sources but the fate of elemental mercury (Hg^0) is of most concern (Driscoll et al. 2007). Atmospheric elemental mercury is oxidized with sun energy to form reactive ionic mercury (Hg^{2+}; Wentz et al. 2014) that then coalesces with atmospheric moisture and is deposited with precipitation onto terrestrial and aquatic ecosystems (Ligocki et al. 1985; Driscoll et al. 2007). Once ionic mercury is deposited into aquatic systems, it undergoes methylation where it is microbially fixed into its most toxic organic form, methylmercury (MeHg), hereon referred to as mercury (Lacerda and Fitzgerald 2001; Benoit et al. 2003; Galloway and Braniireun. 2004). Mercury is concentrated in algal cytoplasm that binds to cellular membranes upon consumption (Mason et al. 1995). This binding property allows mercury to bioaccumulate from trace amounts in algae up through aquatic food webs to potentially toxic levels in large piscivorous fishes (Jackson et al. 1988; Grieb et al. 1990; Mason et al. 1995; Driscoll et al. 2007). Additionally, mercury excretion by organisms is minimal, further facilitating rapid accumulation (Laarman et al. 1976; McKim et al. 1979; Trudel and Rasmussen 1997).

Due to bioaccumulation, high trophic level predatory fishes generally have a high mercury accumulation potential. However, these fishes also tend to be highly sought after for recreational and commercial harvest and consumption, exposing humans to potential mercury contamination. Mercury levels in humans are positively correlated with seafood consumption (USEPA 1997) and excessive consumption of mercury contaminated fish can have detrimental neurological and developmental effects in humans (Murata et al. 2006). Eight percent of pregnant women in the United States have blood mercury levels deemed unsafe by the United States Environmental Protection Agency (USEPA. 1997; Schober et al. 2003) and 410,000 newborn children in the United States are exposed to elevated prenatal mercury levels (Mahaffey

2005). Prenatal mercury exposure due to maternal fish consumption is associated with reduced parasympathetic activity (Murata et al. 2006), reduced neurologic activity, cerebellar ataxia, physical growth disturbance, dysarthria, and limb deformities in children (Marsh et al. 1980; Harada 1995). Severe postnatal mercury exposure is associated with seizures, cerebral palsy, and visual impairment (Harada 1995). Thus, minimizing consumption of fishes with high mercury concentrations would reduce the occurrence of these symptoms.

Global concern of mercury-related health issues has initiated numerous environmental toxin monitoring programs to evaluate mercury levels in a variety of natural resources (e.g., water, fish, birds, etc.) and to promote responsible consumption of seafood resources. In 2001, the United States Environmental Protection Agency (EPA) amended the Clean Water Act under Section 304a, establishing a screening criterion of 0.30 mg of Hg per kg of muscle tissue to aid state agencies in developing fish consumption advisories (USEPA 2010). State agencies are encouraged to establish consumption advisories if fish mercury concentrations are detected above this level (USEPA 2010). As of 2010, 50 states, 1 territory, and 3 Native American tribal governments have established fish consumption advisories for mercury contamination (USEPA 2010). Broadly, mercury concentrations are generally higher in coastal regions with frequent precipitation, such as the Northeastern and Southeastern U.S., compared to inland regions such as the Midwest (Scudder et al. 2009). However, fish mercury concentrations from Midwestern states, such as Iowa, can still reach levels surpassing the EPA criterion (IDNR 2014).

The Iowa Department of Natural Resources (IDNR) initiated the Regional Ambient Fish Tissue (RAFT) Monitoring Program (re-named Iowa Fish Tissue Monitoring Program; IFTMP; in 2015) in 1980 to monitor temporal changes in toxin levels, determine bodies of water containing fishes with elevated toxin levels, and inform anglers who wish to consume fish from

Iowa waters from excessive toxin consumption (IDNR 2014). Under this program, muscle tissue samples from various fish species have been analyzed for polychlorinated biphenyls (PCBs), mercury, chlordane, and other pesticides/herbicides (IDNR 2014). Since 1994, more than 65 of 131 significant public lakes have been sampled for mercury contamination as part of the RAFT program (IDNR, unpublished data). A one meal (6-7 ounces of skinless muscle tissue) of fish per week advisory is issued if mercury concentrations consistently (>2 years) range between 0.3-1.0 mg/kg, whereas a do-not-eat advisory is issued if mercury concentrations consistently exceed 1.0 mg/kg (IDNR 2014). Based on RAFT sampling, a general fish consumption advisory of no more than one meal per week has been issued for twelve lakes and nine river reaches in Iowa (Table 1.1). Eleven of the twelve lakes with consumption advisories are only for largemouth bass (*Micropterus salmoides*), whereas seven of the nine rivers with consumption advisories are for all predatory fishes (Table 1.1). However, other predatory species, including channel catfish (*Ictalurus punctatus*), flathead catfish (*Pylodictis olivaris*), smallmouth bass (*Micropterus dolomieu*), northern pike (*Esox lucius*), and walleye (*Sander vitreus*), have exceeded the 0.30 mg/kg criterion in waters without consumption advisories (IDNR, unpublished data). Lack of advisories on these waters may be due to annual variation in mercury concentrations but are based on samples from a limited number of species and individuals. Further, advisories do not include species-specific threshold lengths at which mercury concentrations warrant consumption advisories (e.g., largemouth bass >XX mm = consumption advisory; Table 1.1). Thus, current advisories may not represent the state of contamination in fishes across all Iowa lakes and rivers.

While monitoring mercury levels in fish is useful for enacting consumption advisories, it provides little insight into mechanisms regulating elevated mercury levels. Fish mercury concentrations can vary greatly between geographically separated fish populations and can be

influenced by a suite of regional, local, and individual scale factors (Larsson et al. 1992; Galloway and Braniireun 2004; Sackett et al. 2009; Hayer et al. 2011). Mercury concentrations in fish depend on three general factors: 1) the amount of inorganic/reactive mercury present in an aquatic system, 2) methylation productivity, and 3) food web structure governing bioaccumulation (Wentz et al. 2014). Many specific abiotic and biotic variables are known to influence these three general factors at the regional, local, and individual scale (e.g., Grieb et al. 1990; Hayer et al. 2011; Sackett et al. 2013).

Although regional variability is common, mercury levels in aquatic systems can also vary greatly between proximal water bodies (Benoit et al. 2003). Both regional and local variation can often be attributed to a suite of watershed-scale factors including watershed size, land use, slope, productivity, wetland area, and water chemistry (Larsson et al. 1992; Lacerda and Fitzgerald 2001; Sackett et al. 2009; Hayer et al. 2011). Fish mercury concentrations also vary within systems due to species, length, age, growth rates, and trophic position (Sackett et al. 2009; Tremain and Adams 2012). Mercury contamination in dietary items (e.g., prey fishes) has also been linked to predatory fish mercury concentrations (Trudel and Rasmussen 2006). Long lived piscivorous fishes, such as northern pike (*Esox lucius*), tend to accumulate elevated mercury concentrations due to their high trophic position in food webs (Olsson 1976; Phillips and Gregory 1979).

Seasonal variation in fish mercury concentrations has also been documented (Ward and Neumann 1999). Fishes sampled in the spring tend to have elevated mercury concentrations compared to summer or fall (Ward and Neumann 1999). This phenomenon could be due to a reduced fat content in muscle tissue during the spring months compared to summer and fall months. Though less likely, this phenomenon could also be due to an increase in runoff from

spring snowmelt and increased rainfall, which provides a seasonal surge of mercury into aquatic systems (Ligocki et al. 1985; Bidleman 1988). Currently, it is unknown if seasonal variation in fish mercury concentrations exist in Iowa and potential seasonal variation is not accounted for in standardized RAFT/IFTMP sampling protocols. Fishes sampled during seasons of low mercury levels may provide a false indication of the maximum accumulation potential. Thus, understanding seasonal fluctuations in mercury levels is an important aspect of successful monitoring programs and may aid the development of standardized fish sampling protocols.

A suite of biotic and abiotic characteristics at regional, local, and individual scales may explain much of the variability in mercury concentrations among fishes in Iowa lakes and rivers. Understanding factors regulating mercury accumulation in fishes is a valuable component of establishing consumption advisories and may provide a tool to predict mercury levels in fishes from other systems, guiding contaminant monitoring programs. Objectives of this project are to (I) evaluate seasonal influences on mercury accumulation in largemouth bass from two Iowa impoundments to assess the need for temporally standardized sampling protocols and (II) evaluate the influence of a suite of biotic (e.g., species, length, age, sex, trophic position) and abiotic (e.g., watershed area, land use, water chemistry) factors on mercury accumulation in fishes in Iowa lakes and interior river systems. Analysis of lakes and rivers are separated into two chapters due to differences in fish species and abiotic and biotic explanatory variables between systems.

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CHAPTER 2

SEASONAL VARIATION OF FISH MERCURY CONCENTRATIONS

Abstract

Mercury contamination in aquatic ecosystems is a global concern due to the health risks of consuming contaminated aquatic organisms, particularly fish. Mercury concentrations in fishes are highly variable and influenced by a range of biotic and abiotic variables. Seasonal variation in mercury levels are typically overlooked when monitoring mercury levels, establishing consumption advisories, or creating accumulation models. Temporally different sampling regimes could bias mercury concentration comparisons and provide biased estimates of accumulation potential. The objectives of this study were to evaluate seasonal variation in largemouth bass (*Micropterus salmoides*) mercury concentrations from two Iowa impoundments, and to evaluate if seasonal variation in mercury concentrations is dependent upon overall mercury contamination or other factors including waterbody type, trophic status, and fish size. Largemouth bass were collected four times per year between May and October (24-36 per month) from Twelve Mile (2013) and Red Haw (2014) lakes, Iowa, USA, with pulsed DC electrofishing. Mercury concentrations in individual fish were highly variable, ranging from undetectable (<0.05 mg/kg) to 0.54 mg/kg. Mercury concentrations were similar across months for Twelve Mile Lake. In contrast, largemouth bass mercury concentrations varied temporally in Red Haw Lake and were highest in July, intermediate in May and September, and lowest during October. Additionally, results of the meta-analysis suggest that seasonal variation in mercury

concentrations is more likely to occur as mean mercury concentration of the population increases but is unrelated to waterbody type, trophic status, and fish size. Understanding seasonal variation in fish mercury concentrations will aid in the development of standardized sampling programs for long-term monitoring and may also play a role in establishing fish consumption advisories.

Introduction

From smallmouth bass (*Micropterus dolomieu*) in the Shenandoah River, VA, U.S.A. (Murphy et al. 2007) to longtail tuna (*Thunus tonggol*) in the Persian Gulf (Saei-Dehkordi et al. 2010), previous studies evaluating seasonal variation in fish mercury concentrations have covered a large breadth of geographic locations, waterbody types, and fish species across the world. Seasonal variation in fish mercury concentrations is not always present (e.g., Farkas et al. 2000; Foster et al. 2000). However, when seasonal variation has been detected, fish muscle tissue mercury concentrations tend to be higher during spring compared to summer or fall (e.g., Ward and Neumann 1999; Farkas et al. 2003; Moreno et al. 2015). However, the majority of studies evaluating seasonal variation in fish mercury concentrations have either been conducted in large European lake systems (e.g., Farkas et al. 2000; Farkas et al. 2003; Moreno et al. 2015) or coastal regions within the United States (e.g., Ward and Neumann 1999; Foster et al. 2000; Greenfield et al. 2013; Kenny et al. 2014). Thus, limited information regarding seasonal variation in fish mercury concentrations is available in Midwestern U.S. regions.

Despite the occurrence of seasonal variation in fish mercury concentrations (e.g., Weis et al. 1986; Ward and Neumann 1999; Kenney et al. 2014), it is typically overlooked when designing mercury monitoring protocols, establishing consumption advisories, or creating accumulation models. Seasonal variation in mercury concentrations has important implications for mercury monitoring programs. Most monitoring programs sample a large number of waterbodies throughout the course of a year and do not account for potential temporal variation. If seasonal variation occurs, asynchronous sampling regimes could bias mercury concentration comparisons among water bodies and provide biased estimates of concentrations at regional,

local, and individual scales. Furthermore, consumption advisories based on models typically do not include temporal variability and may provide inaccurate predictions of mercury concentrations during certain months (Ward and Neumann 1999; Moreno et al. 2015). Synchronizing sampling protocols for mercury in fishes would reduce the effect of temporal variance in mercury concentrations, but is logistically challenging and unnecessary if seasonal variability does not exist. Thus, understanding seasonal variation in fish mercury levels is an important component of successful monitoring programs.

Large piscivorous fishes tend to have elevated mercury levels compared to other fishes at lower trophic levels (Lange et al. 1993). Largemouth bass (*Micropterus salmoides*) are common sport fish and can accumulate mercury concentrations in Iowa surpassing the EPA consumption criterion (IDNR 2014). In Iowa, largemouth bass consumption advisories have been issued for twelve lakes and six rivers, making it a species of contaminant concern. However, standardized temporal sampling protocols have yet to be developed, making it difficult to compare mercury concentrations collected at different times of the year and to develop consumption advisories. Therefore, the objectives of this study were to, 1) evaluate seasonal variation in largemouth bass mercury concentrations from two Iowa impoundments, and 2) conduct a literature meta-analysis to evaluate whether detection of seasonal variation in fish tissue mercury concentrations is dependent upon average mercury contamination or other factors. Understanding seasonal variation in mercury concentrations will aid the development of standardized sampling for long-term mercury monitoring programs.

Methods

Fish Collection & Processing

Largemouth bass were collected four times per year between May and October (24-36 per month) from Twelve Mile (2013) and Red Haw (2014) lakes, Iowa, with pulsed DC electrofishing. Red Haw Lake has a maximum depth of 12.2 m, a mean depth of 4.4 m, a 29 ha surface area, and a 413 ha watershed area. Twelve Mile Lake has a maximum depth of 12.2 m, a mean depth of 4.6 m, a 257 ha surface area, and a 5,931 ha watershed area. Fish of similar length (Twelve Mile: 311-445 mm TL; Red Haw: 278-370 mm TL) were collected to minimize the effect of length as an influential factor affecting mercury concentration. Fish were measured for total length (TL mm) and weight (g) and approximately 1 g of axial muscle tissue was removed from each individual for mercury analysis following EPA fish tissue extraction protocols (USEPA 2000; USEPA 2003).

Equipment (e.g., scalpel, knife, forceps, etc.) used for obtaining tissue samples was sanitized with ethanol to prevent contamination among specimens. Tissue samples were stored in a -10°C freezer until transport for mercury analysis. Within 90 days, tissue samples were transported on ice to the State Hygienic Lab, Ankeny, Iowa, for mercury analysis. Mercury contamination was determined using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) and reported as wet-weight total mercury concentrations (mg/kg; USEPA 1994). USEPA Method 200.8 for determination of mercury concentration procedures was followed to ensure quality assurance and quality control of all samples (USEPA 1994). Mercury detection threshold was 0.05 mg/kg.

Meta-analysis

Three literature searches were conducted to gather published studies evaluating seasonal variation of fish tissue mercury concentrations. For this study, seasonal variation is defined as mean wet-weight muscle tissue mercury concentrations varying within a one year period (365 days from initial collection) for a given fish species collected at least twice in one year. Presence of seasonal variation was noted if the respective statistical test reported by the author(s) was below the significance level of 0.05 (i.e., P-value < 0.05 was noted as statistically significant).

Google Scholar, Web of Science, and EBSCO were searched with the following search phrases: "seasonal variation of fish mercury" and "temporal variation of fish mercury." Between the three search engines, 33 studies evaluating temporal trends of fish tissue mercury concentrations were found. Of the 33 studies found in the literature search, 17 did not meet the criteria due to reporting of dry-weight mercury concentrations, methylmercury concentrations, or use of skin-on fillets. Species-specific data were extracted from 55 fish populations from 16 studies which fit the study criteria (Appendix A). Some studies evaluated seasonal variation of mercury concentrations in multiple species. Extracted data included a binary account of whether or not seasonal variation was detected (i.e., 1 = yes; 0 = no), arithmetic mean mercury concentrations across all seasons, waterbody type (e.g., natural lake, impoundment, river, etc.), a categorical description of trophic level (e.g., piscivore, omnivore, or insectivore), and fish mean total length (mm). If no trophic category was described in the study, diet analyses from Fish Base were used to estimate trophic status (www.fishbase.org; last accessed 10/26/16). In addition to the data extracted from the literature review, information from largemouth bass collected during this study were included in the analyses.

Statistical Analyses

For both Iowa lakes, seasonal variation of largemouth bass mercury concentrations was assessed using Tobit regression (PROC LIFEREG; Statistical Analysis System 9.4; SAS), with the ICP-MS detection threshold of 0.05 as the lower bound, and using Tukey's method for multiple comparisons. Mercury concentrations were log transformed prior to analysis to normalize the residuals. Fish total length was added to each model as a covariate to account for variation due to fish size. A month-length interaction term was initially added to each model to evaluate potential effects of differences in the relationship between fish length and mercury concentration by month. However, these interaction terms were not significant and were therefore omitted from the final analyses. Least squares means was used to obtain estimates of mean mercury concentrations by month. If significant seasonal variation in mercury concentrations existed ($P < 0.05$), differences between months were determined with contrast statements.

Binary logistic regression was used to evaluate the relationship between overall mean mercury concentrations and whether or not seasonal variation of fish tissue mercury concentrations was detected. Additional explanatory variables such as waterbody type, trophic level, and mean total length were added to the model individually to test for significance ($\alpha = 0.05$).

Results

Largemouth Bass in Iowa lakes

For both lakes, largemouth bass mercury concentrations were highly variable, ranging from undetectable (<0.05 mg/kg) to 0.54 mg/kg. However, largemouth bass mercury

concentrations in Twelve Mile Lake were similar among all four months ($P = 0.11$; Table 2.1; Figure 2.1). All fish had detectable mercury concentrations during May but percent of bass with undetectable mercury concentrations increased to 27-32% during the summer and fall months (Table 2.1).

In contrast to Twelve Mile Lake, largemouth bass mercury concentrations varied across months in Red Haw Lake ($P < 0.001$). Mercury concentrations were highest in July, intermediate in May and September, and lowest during October (Table 2.1; Figure 2.2). Percent of undetectable mercury concentrations increased from 0% of May samples to 37% of October samples (Table 2.1). Additionally, mercury concentrations increased with fish total length in the Red Haw Lake ($P < 0.01$; Figure 2.2), but not in the Twelve Mile Lake model ($P = 0.15$).

Meta-analysis

Of the 16 studies identified that evaluated seasonal changes in mercury concentrations in 55 fishes, 38 (69%) found a significant ($P < 0.05$) seasonal variation of fish tissue total mercury concentrations whereas 17 (31%) did not. Logistic regression analysis indicated that the probability of detecting seasonal variation of fish mercury concentration increased with mean mercury concentration of the fishes evaluated ($P = 0.046$; Figure 2.3), but was not related to waterbody type ($P = 0.99$), trophic status ($P = 0.99$), or mean total length ($P = 0.71$). Fish populations with an average mercury concentrations of <0.30 mg/kg have ≤70% probability of detecting seasonal fluctuations, whereas fish populations with an average mercury concentration of >0.30 mg/kg have over a 70% probability of experiencing seasonal fluctuations in mean mercury concentrations (Figure 2.3). In addition, 90% of studies exceeding an overall mean mercury concentration of 0.30 mg/kg found significant seasonal variation.

Discussion

Although average mercury concentrations were similar in the two study lakes, seasonal variation of largemouth bass mercury concentrations was only detected in one lake. Various studies have shown fish mercury concentrations to peak during the spring and then decline throughout the summer and fall months (e.g., Meili 1991; Ward and Neumann 1999; Bratten et al. 2014; Kenney et al. 2014). Contrary to this phenomenon, largemouth bass mercury concentrations in Red Haw Lake were found to peak during mid-July, with intermediate levels in September and the lowest levels observed in October.

While there was a statistical difference in largemouth bass mercury concentrations among months in Red Haw Lake, the maximum mean difference between July and October was only 0.12 mg/kg. Thus, fish mercury sampling regimes may not need to be temporally standardized. Additional seasonal sampling of other lakes throughout the Midwest region with elevated mercury levels may help to add clarity to the extent to which seasonal variation of fish mercury concentrations exists in the Midwestern United States.

Largemouth bass mercury concentrations observed in this study were 2-4 times lower than other studies evaluating seasonal variation in black bass (*Micropterus spp*.) mercury concentrations (e.g., Ward and Neumann 1999; Foster et al. 2000; and Murphy et al. 2007). Thus, based on the results of the meta-analysis, the absence of seasonal variation and the subtle seasonal variation detected in this study may be in part due to a relatively low average mercury concentration. Although average mercury concentration may be a factor influencing whether or not seasonal variation exists, this simple measure likely overlooks various mechanisms that may also influence whether or not seasonal variation exists.

There have been several explanations proposed as to why seasonal variation of fish mercury concentrations occurs. First, seasonal warming of water temperature may cause an increase in methylation by mercury-fixing microbes, resulting in an increase in bio-available mercury (Weis et al. 1986). Second, a seasonal increase in spring rains may be a source for aerial deposition of mercury (Weis et al. 1986). Third, seasonal variation in fish feeding rates, such as an increased pre-spawn feeding rate could result in a pulse of mercury consumption via prey items (Weis et al. 1986). Ward and Neumann (1999) suggested seasonal variation in dominant food items as a potential mechanism driving seasonal variation in fish mercury concentrations. However, because bioaccumulation of mercury is a time-integrated process, a relatively short pulse of mercury into aquatic systems/organisms, such as a spring rains or a brief increase in feeding rate, would not likely immediately increase fish muscle tissue mercury concentrations. Additionally, fish feeding rates are generally high throughout the growing season (Cochran and Adelman 1982), and excretion of mercury is extremely low (Laarman et al. 1976). Thus, a brief shift in prey items would probably not result in a decline in fish mercury concentrations throughout the summer and fall months.

Perhaps a more realistic explanation for seasonal variation in fish tissue mercury concentrations would be the proximate composition of muscle tissue (composition of moisture, ash, lipids and proteins; Ward and Neumann 1999). Methylmercury binds to sulfhydryl groups on proteins, and not lipids (Laarman et al. 1976). Thus, fish muscle tissue with low percent lipid composition should have a higher mercury concentrations compared to similar fish muscle tissue with a high percent lipid composition. Fish muscle lipid composition tends to be lower during the spring months, when lipid stores have been depleted throughout the winter (Leu et al. 1981; Weatherly and Gill 1987; Bae and Lim 2012; Kailasam et al. 2015). Conceptually, the proximate

composition of the fish muscle tissue is slowly enriched with lipids throughout the growing season (Griffiths and Kirkwood 1995), diluting the protein mass in the muscle tissue and corresponding mercury concentrations per unit wet weight.

Despite these processes, monthly variations of mercury concentrations in horse mackerel (*Trachurus trachurus*) and Atlantic bonito (*Sarda sarda*) are positively related to lipid content and a negatively related to protein content (Özden 2010). However, mercury concentrations were not adjusted for fish length or age, which can have a substantial influence on mercury concentrations and may have confounded these relationships (Weiner and Spry 1996; Tremain and Adams 2012). In another study evaluating mercury in freshwater fishes, Griffiths and Kirkwood (1995) found fat content of roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) to increase steadily over the growing season, which may have implications for protein mass dilution. Conversely, this field study took place in a region with distinct growing and non-growing seasons, and one of the study lakes was not found to have seasonal variation of fish tissue mercury concentrations.

Results of this study indicate that largemouth bass mercury concentrations varied seasonally in one of the study lakes but not the other, suggesting mercury concentrations can fluctuate seasonally in some water bodies, but not others even in close proximity. Seasonal variation of fish mercury concentrations was related to the overall level of contamination. My results suggest that seasonal variation may be prevalent in populations where the annual mean concentration is >0.30 mg/kg. Thus, seasonal sampling to detect this potential variation may be warranted. Seasonal sampling of fishes for mercury monitoring can substantially increase effort and monetary costs and would target fish populations with greater health concerns (USEPA 2010). Further evaluations of seasonal variation of fish mercury concentrations, particularly of

populations exceeding 0.30 mg/kg, would help to confirm the relationship found in this study between the probability of detecting seasonal variation and overall mercury contamination.

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Tables and Figures

Table 2.1. Largemouth bass sample size (n), mean mercury concentrations (mg/kg; ± 95% confidence interval), percent of largemouth bass sampled with undetectable mercury concentrations (<0.05 mg/kg), and mean bass total length (mm) sampled from Twelve Mile and Red Haw lakes, May-October 2013 and 2014, respectively. Within each lake, means sharing a common superscript are not significantly different ($\alpha = 0.05$).

Lake	Month	n	Mean TL (mm)	Mean Hg ± 95% C.I.	% <0.05 mg/kg
Twelve Mile Lake	May	31	373	0.19 ± 0.05^{a}	0
	July	27	374	0.12 ± 0.04^{a}	33
	August	23	382	0.12 ± 0.04^{a}	30
	October	33	380	0.14 ± 0.04^{a}	27
Red Haw Lake	May	30	338	0.17 ± 0.03^{a}	0
	July	33	310	0.23 ± 0.04^{b}	3
	September	36	315	0.16 ± 0.03^{a}	11
	October	30	312	0.11 ± 0.02^{c}	37

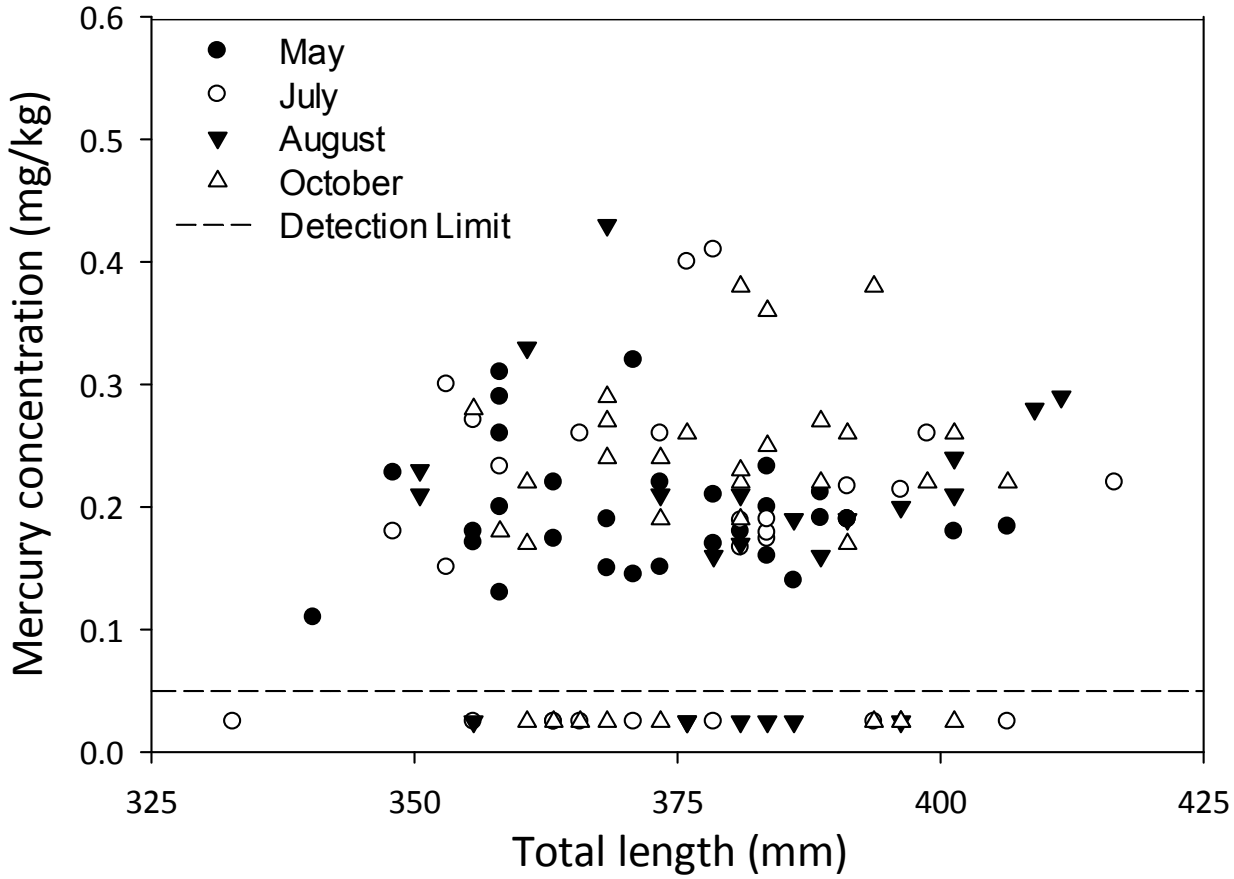

Figure 2.1. Relationship between largemouth bass total length (mm) and total mercury concentrations (mg/kg) during May (●), July (○), August (▼), and October (△) for Twelve Mile Lake, Iowa, 2013. Dashed line represents the detection limit (0.05 mg/kg).

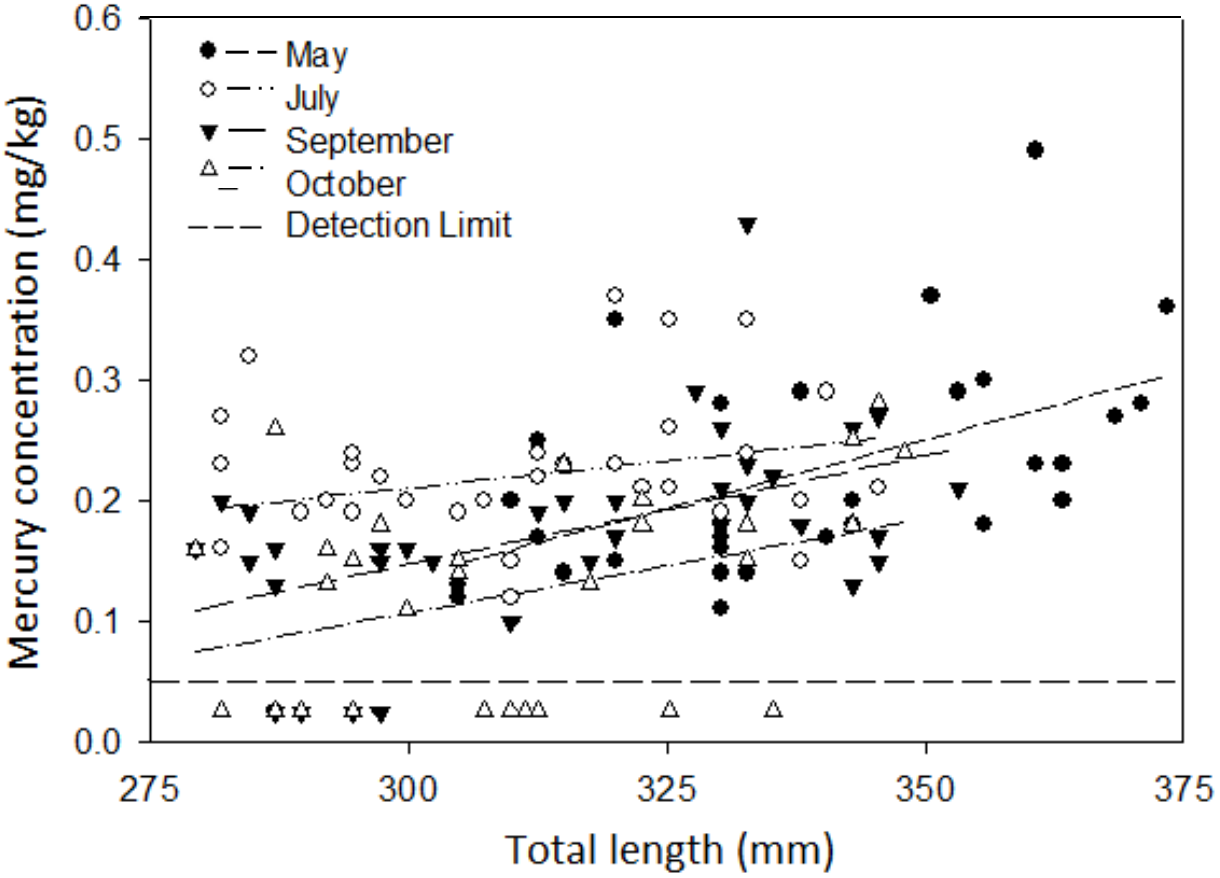

Figure 2.2. Relationship between largemouth bass total length (mm) and total mercury concentrations (mg/kg) during May (●), July (○), September (▼), and October (△) for Red Haw Lake, Iowa, 2014. Horizontal dashed line represents the detection limit (0.05 mg/kg).

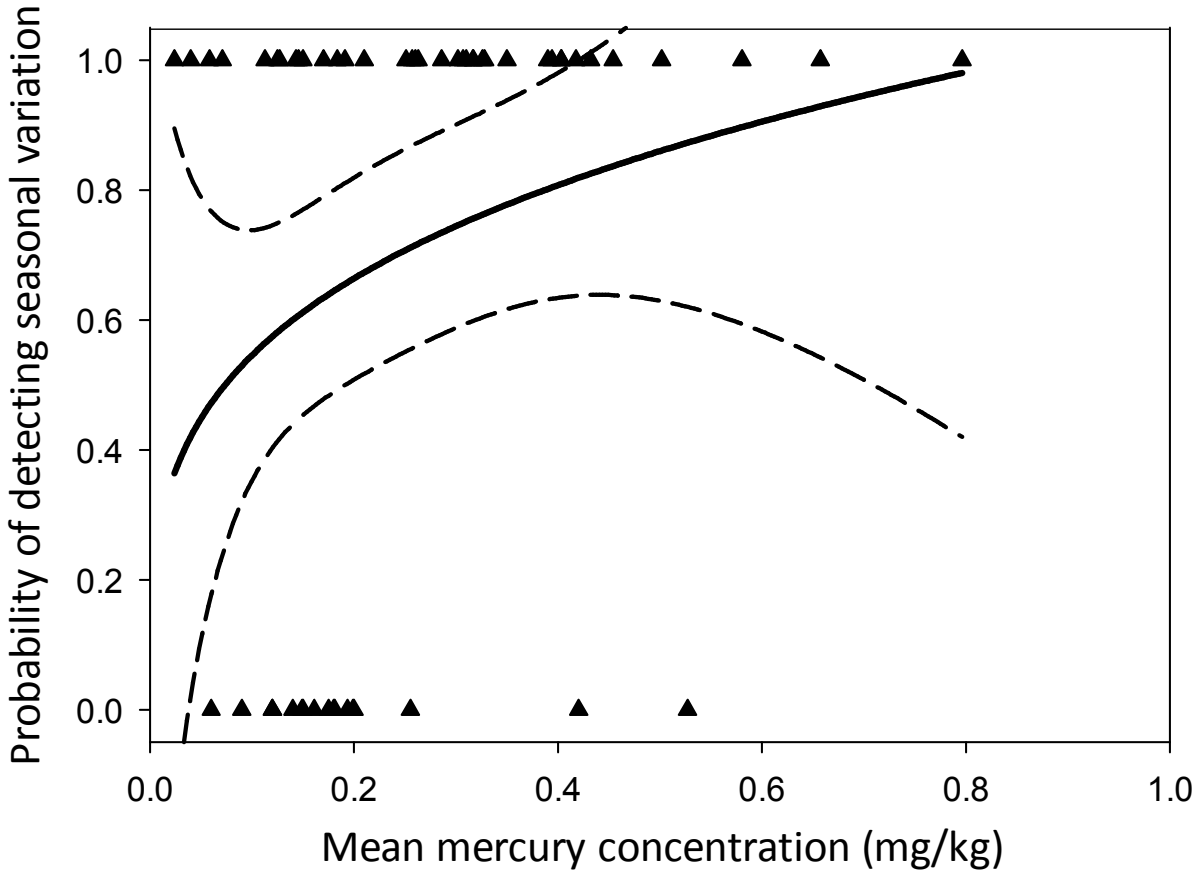

Figure 2.3. Binary logistic curve fitted to points evaluating the relationship between mean mercury concentrations in fish muscle tissue and seasonal variation in mercury concentrations. 1 = seasonal variation was detected, 0 = seasonal variation was not detected. Dashed lines represent 95% confidence bands.

CHAPTER 3

FACTORS INFLUENCING FISH MERCURY CONCENTRATIONS IN IOWA LAKES

Abstract

Mercury contamination in aquatic ecosystems is a global concern due to the health risks of consuming contaminated aquatic organisms, particularly fishes. Mercury concentrations in fishes are highly variable within and among systems, and are influenced by a range of biotic and abiotic variables. However, predictive mercury models are region specific and factors influencing fish mercury concentrations across Iowa lake systems are unknown. Bluegill (*Lepomis macrochirus*, n = 275), white and black crappie (*Pomoxis annularis*, n = 112; *P. nigromaculatus*, n = 203), largemouth bass (*Micropterus salmoides*, n = 502), walleye (*Sander vitreus*, n = 248), muskellunge (*Esox masquinongy*, n = 30), and northern pike (*E. lucius*, n = 45) were collected between April and October, 2013-2015, from natural lakes (n = 6), shallow natural lakes (n = 2), constructed lakes (n = 18), and reservoirs (n = 4) throughout Iowa and tested for mercury contamination. Various land use, water chemistry, and fish characteristics were gathered and used to explain differences in mercury concentrations across and within lake systems. Mercury concentrations of Iowa fishes were generally low, and the concentration in many fish was undetectable (<0.05 mg Hg/kg; 43% of observations). Thus, multiple linear regression was first used to evaluate factors related to detectable mercury concentrations. Second, logistic regression was used with detected and undetected observations to predict the probability of detecting mercury concentrations. Detected mercury concentrations were highest

in muskellunge, northern pike, walleye and largemouth bass, lowest in black and white crappie and bluegill, and positively related to fish length and age in all species. Though not significantly different across all species, females generally had slightly higher mercury concentrations than males. Additionally, pH, lake mean depth, watershed area to lake area ratio, and percent of watershed as forested land, grasslands and open water were positively related to fish mercury concentrations, whereas lake area, and percent of watershed as agriculture and developed land were negatively related to mercury concentrations. Finally, mercury concentrations were on average 28% higher in shallow natural lakes compared to other lake types. Together, these factors explained 74% of the variation in detectable fish mercury concentrations. Second, the logistic model correctly predicted the probability of detecting mercury concentrations for 91% of the 1,415 fish sampled. This study provides a comprehensive analysis of abiotic and biotic factors influencing fish mercury concentrations in Iowa lakes and may have implications for refining consumption advisories.

Introduction

Fish consumption has become a global concern due to the health risks of mercury contaminated fish. Consumption of mercury-contaminated fishes is associated with a range of neurological and developmental disorders in humans (Harada 1995; Murata et al. 2006). Mercury monitoring programs typically provide information about mercury concentrations in some species, but seldom address mechanisms of accumulation. Mercury originates from both natural and anthropogenic sources (Pirrone et al. 2010). Environmental mercury levels have increased since the 19th century due to anthropogenic sources, particularly fossil fuel combustion (Driscoll et al. 2007; Mason 2009; Pirrone et al. 2010). Among other chemical forms of mercury, elemental mercury is released into the atmosphere upon combustion of coal and fossil fuels (Driscoll et al. 2007) where it coalesces with water molecules and is precipitated across terrestrial and aquatic landscapes (Ligocki et al. 1985; Driscoll et al. 2007). Once leached or deposited into aquatic systems, mercury is methylated via mercury-fixing bacteria into its most toxic organic form, methylmercury, hereon referred to as mercury (Lacerda and Fitzgerald 2001; Benoit et al. 2003; Galloway et al. 2004). Mercury then bioaccumulates in aquatic food chains and can be found at toxic concentrations in a variety of fishes (e.g., Jackson et al. 1988; Grieb et al. 1990; Mason et al. 1995).

Regional variability in mercury levels can be dependent on watershed-scale factors influencing methylation. Mercury concentrations in fish generally increase with watershed area likely due to the amount of mercury available for methylation (Rypel 2010; Hayer et al. 2011). Within a watershed, land use can influence mercury accumulation by affecting water quality and productivity (Wood 1980; Wren and Macrimmon 1983; Benoit et al. 2003). Mercury-fixing

bacteria are concentrated in wetland areas (Driscoll et al. 2007), resulting in elevated mercury levels in systems with high wetland coverage (Rypel 2010). Additionally, areas with large amounts of agricultural fertilizer runoff contribute excess nutrients to systems and increase productivity (Correll 1998). Lake productivity and associated Trophic State Index (TSI) can be negatively related to mercury accumulation in fishes (Larsson et al. 1992; Pickhardt et al. 2002; Rypel 2010). Fish populations in highly eutrophic lakes can experience faster growth rates leading to growth biodilution of mercury compared to fishes inhabiting less productive systems where slow growth rates lead to concentrated bioaccumulation (Pickhardt et al. 2002). Further, fish mercury concentrations have been related to water chemistry variables, such as pH (Wood 1980). Acidic lakes have relatively low pH that is related to increased microbial activity, including mercury-fixing bacteria (Wood 1980) resulting in fish mercury concentrations being negatively related to water pH (Wren and MacCrimmon 1983; Driscoll et al. 1994; Hakanson 2003). In addition to abiotic influences, piscivorous fishes tend to bioaccumulate higher levels of mercury compared to omnivorous or planktivorous fishes (Olsson 1976; Mason et al. 1995; Wiener and Spry 1996) with mercury concentrations positively related to fish length and age (Tremain and Adams 2012).

Predictive mercury accumulation models have identified various factors that help explain mercury variability among fishes (e.g., Sackett et al. 2009; Hayer et al. 2011). Models are important aspects of successful mercury monitoring programs because mercury contamination can be predicted in water bodies that have not been sampled. For instance, fish trophic position, species, ecoregion, and water pH explained 81% of mercury variation in North Carolina freshwater fishes (Sackett et al. 2009, 2013). Using a similar model, Hayer et al. (2011) found water quality attributes to be poor predictors of walleye mercury concentrations in South Dakota,

while watershed-level characteristics (watershed slope, lake level changes, agricultural area, wetlands) explained up to 81% of the variation in mercury concentrations. Thus, predictive mercury accumulation models appear to vary among locations, limiting their extrapolation to other regions.

Regional, local, and individual differences in abiotic (e.g., watershed size, land use, water chemistry, etc.) and biotic (e.g., length, age, species) characteristics may explain much of the variability in mercury concentrations in fishes. They may also play a large role in constructing predictive mercury accumulation models for application to regionally similar lakes that have not been evaluated. The objective of this study was to explore the influence of a suite of abiotic and biotic factors on mercury concentrations in freshwater fishes from Iowa waterbodies. I hypothesized that variation in fish mercury concentrations would be explained by multiple abiotic and biotic factors, including water chemistry, lake morphology, watershed characteristics and land use, and fish characteristics. Results from this study provide useful predictive and descriptive information regarding variation in fish mercury concentrations throughout Iowa.

Methods

Fish Collection & Processing

Bluegill, white and black crappie (*Pomoxis annularis, P. nigromaculatus*), yellow perch (*Perca flavescens*), largemouth bass (*Micropterus salmoides*), walleye (*Sander vitreus*), muskellunge (*Esox masquinongy*), and northern pike (*E. lucius*) were collected between April and October, 2013-2015, from natural lakes (n = 6), shallow natural lakes (n = 2), constructed lakes (n = 18), and reservoirs (n = 4) throughout Iowa (Figure 3.1; Table 3.1). Shallow natural

lakes are generally defined by a mean depth of <3 meters, compared to other natural lakes which have greater mean depths (Burks et al. 2006). Constructed lakes are generally small (< 500 ha) manmade impoundments created for fishing and recreation purposes. Reservoirs are generally large (>1000 ha) and outflow is controlled by the United States Army Corps of Engineers to regulate flow in the Mississippi and Missouri rivers.

Fish were collected with pulsed DC electrofishing, experimental gill nets, and modified fyke nets. A minimum of eight individuals of each species were collected by ~1 cm length groups (i.e., 1 individual/species/length group) from each system. Fish were measured for total length (TL mm) and weight (g) and one fish per species and 1 cm length group was euthanized. Fish not processed immediately after capture were wrapped in aluminum foil, labeled with weight and length measurements, and frozen whole until processing. In the laboratory, sex (male, female, or unknown) was determined and aging structures applicable to each species were removed. All tissue samples were collected following United States Environmental Protection Agency (USEPA) fish tissue extraction protocols (USEPA 2000; USEPA 2003). One 5-10 g sample of skinless dorsal axial muscle tissue was removed from each individual for mercury analysis. Tissue samples were removed wearing nitrile gloves and with a scalpel. Gloves were replaced and scalpels were thoroughly sanitized with 95% ethanol after each fish to avoid cross-contamination among samples. Tissue samples were stored in a -10°C freezer until transport for analysis. Frozen fish tissue samples were transported on ice to the State Hygienic Lab, Ankeny, Iowa, for mercury analysis. Mercury contamination was determined using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) and reported as wet-weight total mercury concentrations (THg mg/kg; USEPA Method 200.8; USEPA 1994). USEPA). Mercury detection threshold was >0.05 mg/kg.

Otoliths were the primary aging structure for bluegill, crappie, largemouth bass, and walleye (used for 99% of individuals), but spines and scales (1% of individuals), were used when otoliths were not readable. Pelvic fins were used to age northern pike and muskellunge (Brenden et al. 2006). Otoliths and spines were cross-sectioned (0.8 – 1.0 mm thickness) using a slow speed saw with a diamond wafering blade. Structures were aged at least two times by one reader without prior knowledge of fish size or capture location. Additional cross-sections were taken and ages were re-estimated when there were disagreements among age estimates.

Limnological & Watershed Data

Water quality and water chemistry data were extracted from the Iowa Department of Natural Resources (IDNR) Iowa Lakes Information System (ILIS) online database (http://limnology.eeob.iastate.edu/lakereport/default.aspx; last accessed 8/01/2016). Water quality attributes extracted from this database included Secchi depth (m; SD), dissolved organic carbon (mg/kg; DOC), turbidity (NTU; Turb), chlorophyll *a* (µg/L; Chl *a*), total phosphorous (µg/L; TP), total Kjeldahl-nitrogen (mg/L; TKN), pH, alkalinity ($CaCO_3$ mg/L), total volatile suspended solids (mg/L; TVSS), total suspended solids (g/L; TSS), and Carlson Trophic State Index (TSI; Carlson 1977) values based on Secchi depth, chlorophyll *a*, and total phosphorous (Appendix B). Water samples were collected 1-3 times per year between 2000 and 2015 by the Iowa Lake Monitoring Program. Water quality variables were averaged based upon the age of each fish in each waterbody and are considered fish-level variables for this study. For example, a 4-year-old fish collected during 2015 had water quality metrics averaged for years 2011-2015.

Lake-level data that included watershed land use data and lake morphometric characteristics were extracted from the IDNR Iowa Lakes Mapping and GIS Library databases

(https://programs.iowadnr.gov/nrgislibx/; last accessed 10/27/2016). Data was compiled for all 30 lakes and included maximum depth (m; MxD), mean depth (m; MD), lake area (ha; LA), lake volume (m^3; LV), watershed area (km^2; WA), watershed area to lake area ratio (WA.LA) and watershed composition data including open water (%; perWater), wetland area (%; perWet), grassland area (%; perGrass), forested land (%; perFor), row crop agriculture (%; perAg), and developed land (%; perDev). East Okoboji and West Okoboji are openly connected, allowing fish passage between the two lakes, and are considered one lake for management purposes (e.g., stocking). Therefore, fishes collected from East Okoboji were assigned limnological data that was averaged across East and West Okoboji. Watershed data for West Okoboji was used for all observations because it is further down in the drainage basin. Additionally, the frequency of hypoxic conditions was available for each lake and is defined as the frequency of observing 2 mg/L or less of dissolved oxygen in the deepest part of the water column (Freq.hyp).

Statistical analyses

The original set of explanatory variables was reduced by eliminating correlated variables that represent similar attributes (Appendix C). Variables eliminated during this process included Secchi depth, chlorophyll-*a*, total phosphorous, total Kjeldahl nitrogen, volatile suspended solids, total suspended solids, lake volume, and lake maximum depth. Total Kjeldahl-nitrogen, Secchi depth, chlorophyll-*a*, and total phosphorous were all correlated with TSI and represent similar measures of lake productivity (Appendix C); thus, only TSI was retained for analysis. Turbidity, volatile suspended solids, and total suspended solids were all correlated and represent measures of particulates in the water column. Turbidity has been used previously in predictive mercury model evaluations (e.g., Sackett et al. 2009) and was retained. Additionally, lake

volume was correlated with and is a function of lake mean depth and lake surface area, and therefore was eliminated. Lake maximum depth and mean depth were highly correlated. However, mean depth better represents a lake depth profile compared to maximum depth that was eliminated because it represents a single point on a lake. This process resulted in the retention of 17 environmental variables for further analysis. Additionally, due to a low sample size of detected mercury concentrations (n = 3 of 44 fish collected), yellow perch were omitted from the analysis. Finally, due to similarities in biological traits and mercury concentrations, white and black crappie were combined into a single species category (crappie; CRP) for this analysis.

Differences in environmental characteristics among lakes (water chemistry [15 year average], lake morphometry, and watershed composition data) were explored by using nonmetric multidimensional scaling (NMDS) ordination. The 17 environmental variables were first normalized (mean = 0, standard deviation = 1), a 30 x 30 Euclidean Distance matrix was calculated, and finally the matrix was used as input to the NMDS ordination. A second ordination of lakes was conducted using only 10 environmental variables identified to influence fish mercury concentrations based on a model selection procedure. Lakes were plotted by lake type and ecoregion to show similarities among lakes of similar types and within ecoregions with vectors labelled along the axes indicating variables most strongly associated with spatial orientation within the ordination space. Only variable vectors that were significantly correlated with NMDS axes scores ($r \geq 0.50$, $P < 0.05$) are labeled on the axes. Using the second ordination, additional plots were created to show relative differences in mean mercury concentration between lakes as different sized points in ordination space. The normalization,

distance matrix, NMDS ordination, and vectors were generated using PRIMER (Clarke and Gorley, 2006).

A large portion of fish in the dataset had undetectable mercury concentrations (43%). Interval Censored Regression (ICR) has been used to account for high numbers of undetected values (e.g., Glover et al. 2010). However, ICR, and many other censored models assume that undetected values are derived from the same distribution as detected values. Undetected mercury concentrations observed in this study, particularly in piscivorous fishes such as largemouth bass, northern pike, and walleye, appeared more random than from the same distribution of detected mercury concentrations (Figure 3.2). To evaluate if undetected concentrations were derived from the same distribution as detected data, a preliminary full multiple regression model (i.e., all abiotic and biotic predictor variables in dataset and with no interaction terms) was created using only the detected values. The resulting model regression coefficients were then used to predict mercury concentrations in undetected observations. I hypothesized that if the full model predicted low concentrations (close to or below the detection limit of 0.05) for undetected observations, then the data are from the same distribution. If the full model predicted a range of concentrations exceeding the detection limit, then the detected values and undetected values were likely to be derived from separate distributions.

The model only predicted 1 of the 645 undetected mercury concentrations to be below the detection limit. Thus, the use of ICR to account for undetectable concentrations was not appropriate. Instead, a multiple linear regression model using only detected observations was used to predict fish mercury concentrations. Second, a logistic regression model derived from detected and undetected observations was used to predict the probability of detecting mercury concentrations. This two-part model predicts two valuable pieces of information. First, the

logistic model predicts the probability that mercury was detected in the fish tissue. Second, the multiple linear regression model predicted detected fish tissue mercury concentration based on the variables identified in the model selection procedure. Finally, analysis of variance (ANOVA) was conducted on detected mercury concentrations for all categorical variables identified to influence fish mercury concentrations.

Building the predictive model

I used a model selection procedure to determine the best predictors of detected mercury concentrations. For this step, I only used detected mercury concentrations to build the final model, using a regression subset selection procedure, hereon referred to as "regsubsets", under the R-package, "leaps" (Thomas Lumley using Fortran code by Alan Miller 2009; R Core Team 2016). In addition to the 27 individual abiotic and biotic variables of interest (Appendix B), I added interaction terms between species-age, species-sex, species-length, and sex-length to allow for different slopes between these variables and fish mercury concentrations. A value of zero was used for years since construction for natural lakes and shallow natural lakes because they are not constructed. Therefore, an interaction between lake type and year-since-construction was necessary to allow a different slope for constructed and reservoir lakes compared to natural lakes and shallow natural lakes. Fish mercury concentrations, watershed area to lake area ratio, watershed area, and all percent watershed composition variables were log-transformed prior to analysis to normalize residuals.

The model selection procedure was conducted in two parts; fish-level variables (biotic and water chemistry data) and lake-level variables (lake morphometric characteristics and watershed composition data). First, regsubsets was conducted on fish-level variables with a fixed

effect on ‘Waterbody’. The Waterbody variable is a term unique to each lake (lake name) and is used to account for variation in fish mercury concentrations among waterbodies while regsubsets selects models that explain the most variation in fish mercury concentrations using fish-level variables. All possible model combinations were run and models were sorted using Akaike’s Information Criterion (AIC_c). Variables and interactions included in the top model were retained when evaluating lake-level variables. All other fish-level variables were removed from the model for the succeeding steps in the analysis.

Next, I ran another exhaustive regsubsets model selection procedure on lake-level variables to determine if any additional variation in fish mercury concentrations could be explained by lake specific characteristics. For this step, the “Waterbody” term was omitted and the retained fish-level variables, determined in the previous step, were forced into the model selection procedure. Using the combined results of the two model selection procedures, a final multiple linear regression model was created to describe variation in fish mercury concentrations within and across lakes.

Logistic analysis

Binary logistic regression was used to determine the probability of detecting fish mercury concentrations based on the predictors determined in the final full model using both detected and undetected concentrations. I assumed the same factors influencing detected fish mercury concentrations would influence undetected mercury concentrations. Therefore the same variables and interactions were used to create the logistic model. The reliability of the logistic model was tested by determining the percent prediction error rate (i.e., how often the model correctly predicts whether or not a fish will have a detected mercury concentration). This involved using

the logistic model to predict the probability of detecting mercury concentrations using the same data that was used to create the model, and then comparing the accuracy of the predictions to whether or not mercury was actually detected. Predictions at or above 0.50 (≥50%) were classified as detected, and predictions below 0.50 were classified as undetected.

Results

Ordination #1

Two-dimensional ordination of lakes based on 17 environmental variables had a stress value of 0.18, indicating the ordination provides a useful picture of similarities and differences among lakes, but that the precise locations of lakes in ordination space should be interpreted with caution (Clarke and Warwick 2001). With the exception of Rathbun Reservoir (18), the four lake types grouped together in ordination space indicating similarities across the 17 environmental variables (Figure 3.3). Rathbun Reservoir was positioned in the upper middle of the ordination reflecting several characteristics more in common with constructed lakes than the other three reservoirs (Figure 3.3). The two shallow natural lakes, Little Wall Lake (12) and Crystal Lake (8), grouped in the lower left corner of the ordination based primarily on their shallow mean depths, low percentages of grasslands and agriculture in their watersheds, and high trophic state and turbidity (Figure 3.3). With the exception of Okoboji lake (16), the six natural lakes grouped in the lower middle of the ordination based primarily on their average percentages of agriculture and grassland in their watersheds, high alkalinity, and northern locations (Figure 3.3). The eighteen constructed lakes grouped in the upper middle of the ordination based primarily on their lower trophic state and turbidity, higher percentages of forest and grassland in their watersheds,

lower percentages of agriculture, lower alkalinity, and more southerly locations (Figure 3.3). Three of the four reservoirs, Coralville, Saylorville and Red Rock, were positioned at the right of the ordination primarily reflecting their large areas, watershed areas, and watershed to lake area ratios (Figure 3.3).

When plotted by ecoregion the ordination showed less cohesiveness within groups than when plotted by lake type, but some patterns were evident (Figure 3.3). Lakes in the DSML were distributed across the lower right half of the ordination based primarily on their low percentages of grassland and high percentage of agriculture in their watersheds, high alkalinity, trophic state and turbidity, and their northerly locations (Figure 3.3). Lakes in the SIRLP and CIP were distributed across the upper half of the ordination based on characteristics opposite those of lakes in the DSML (Figure 3.3).

Fish-level factors

Overall, mercury concentrations of Iowa fishes were generally low, and the final data set contained a high percentage of undetectable concentrations (<0.05 mg/kg; 43% of observations). Bluegill, yellow perch, and crappie had the highest percentages of undetected mercury concentrations, ranging from 69-93% (Table 3.2). Likewise, these three species had the lowest mean detected mercury concentrations, ranging from 0.08-0.10 mg/kg (Table 3.2). Bluegill, largemouth bass, and crappie were collected from the highest number of lakes (26; Table 3.2). However, some lakes had only undetected mercury concentrations for bluegill and crappie (Table 3.3), reducing the number of lakes included in the analysis to only 7 for bluegill and 18 for crappie (Table 3.4).

Mean mercury concentrations were highest in muskellunge, northern pike, and largemouth bass (Table 3.2; ANOVA; $P < 0.01$). The highest observed mercury concentration, 2.52 mg/kg, was detected in a 1204 mm, 14-year-old female muskellunge from East Okoboji Lake in northwest Iowa. However, relatively few muskellunge were collected from Iowa lakes (n = 30).

According to the results of the fish-level model selection procedure, the top multiple regression model ($\Delta AIC_c = 0$; $w_i = 0.27$) included fish total length, species, sample weight, age, sex, and Julian day (Table 3.5). All top ten models included fish total length, sample weight, species, and fish age, suggesting these variables were influencing fish mercury concentrations (Table 3.5; Figure 3.5).

Fish mercury concentrations were positively related to length and age (Table 3.2; Figures 3.2, 3.5, and 3.6). Mercury concentrations were related to fish length for all species except crappie (Table 3.2), whereas all species except northern pike were related to fish age (Table 3.2). Age was a better predictor (higher R^2 value) of mercury concentrations than total length for muskellunge, crappie, and walleye (Table 3.2).

Mean male and female mercury concentrations across all observations were similar to one another (ANOVA; $P = 0.23$; Table 3.6; Figure 3.7). The main effect of sex was not in the top model, but the interaction between sex and length was (Table 3.5). The regression slopes for males compared to females were generally smaller when separated by species (Figure 3.6). However, differences between sexes are marginal for most species (Table 3.6; Figure 3.6).

In addition to biotic fish characteristics, pH and dissolved organic carbon were retained in the final model. pH was positively related to fish mercury concentrations but the slope of dissolved organic carbon was not different from zero (Table 3.7; Figure 3.5). In contrast, the

effects of TSI and alkalinity received little support and were not retained in the final predictive model (Tables 3.5). Finally, in addition to water chemistry, Julian day and sample weight were negatively related to fish mercury concentrations (Table 3.7; Figure 3.5).

Lake-level factors

According to the results of the lake-level model selection procedure, the top multiple regression model ($\Delta AIC_c = 0$, $w_i = 0.33$) contained lake area, mean depth, lake type, ecoregion, watershed area to lake area ratio, and percent open water, forest, grassland, agriculture, and developed (Table 3.8). Though not retained in the top AIC_c model, percent wetland area, northing, easting, and frequency of hypoxia were retained in many other models that received support ($\Delta AIC_c < 2$, $w_i > 0.10$; Table 3.8).

Parameter estimates in the final predictive model suggest fish mercury concentrations were negatively related to lake area, but positively related to mean depth and watershed to lake area ratio (Table 3.7; Figure 3.8), suggesting deep lakes with small surface area and watershed to lake area ratios tend to have the highest fish mercury concentrations. Northing and easting coordinates were not retained in the top AIC_c model (Table 3.8). However, a slight pattern of increasing fish mercury concentrations from northwest to southeast can be seen when plotted geographically, but is best depicted with mean largemouth bass mercury concentrations (Figures 3.9-3.12).

Fish mercury concentrations varied among a few of ecoregions (ANOVA; $P < 0.05$). Highest mean mercury concentrations were found in the CIP ecoregion and lowest fish mercury concentrations found in the DSML, NILP, and SIRLP ecoregions (Table 3.9; Figure 3.13). Additionally, fish mercury concentrations varied across lake types, with highest mean mercury

concentrations found in shallow natural lakes and lower concentrations found in reservoirs, constructed lakes, and natural lakes (Table 3.10; Figure 3.14; ANOVA; $P < 0.05$).

Percent open water, forested land, grassland area, agriculture, and developed land were in the top model (Table 3.8). Parameter estimates in the final predictive model suggest percent open water is positively related to fish mercury concentrations, while percent forested land, grassland area, agriculture, and developed land are negatively related to fish mercury concentrations (Table 3.7; Figure 3.15). However, bivariate relationships between mean fish mercury concentrations by lake and percent forest and percent grassland area indicate a positive relationship (Figure 3.15). This discrepancy is likely due to other variables in the model influencing the parameter estimates of these variables.

Ordination #2

The second ordination constructed using the 10 environmental variables identified to influence fish mercury concentrations had a stress value of 0.17, indicating that this ordination also provides a useful picture of similarities and differences among lakes (Figure 3.16; Clarke and Warwick 2001). Variables influencing NMDS axes 1 and 2 were similar to those from the first ordination, resulting is a similar positioning of lakes within the ordination space (Figure 3.3 and 3.16). Mean mercury concentrations in all species were variable throughout the ordination space and no strong pattern emerged (Figures 3.9-3.12).

Model predictions

The reduced final model contained 17 variables that explained 74% of the variation in fish mercury concentrations collected from Iowa lakes ($R^2 = 0.74$; Figure 3.17). Additionally, the

prediction error rate for the logistic analysis was 11%, indicating the logistic model correctly predicted the probability of detecting mercury concentrations for 89% of the 1415 observations (Tables 3.11). Model parameter estimates indicated that the probability of detecting mercury increased with sample weight, fish age, pH, mean depth, watershed to lake area ratio, and percent water and grassland and decreased with Julian day, lake area, and percent agriculture (Table 3.12).

Discussion

Results from this study suggest that a suite of biotic and abiotic factors are associated with differences in fish mercury concentrations. With varying degrees of effects, fish length, species, sex, age, sample weight, Julian day, pH, mean lake depth, lake area, lake type, ecoregion, watershed area to lake area ratio, and land use explained 74% of the variation in fish mercury concentrations in Iowa lakes. Similar to previous evaluations (e.g., Phillips et al. 1980; Eagles-Smith et al. 2008; Sackett et al. 2009), larger and more piscivorous fishes, such as muskellunge, northern pike, and largemouth bass tended to have the highest mercury concentrations. However, because of potential growth biodilution, age may be a better predictor of fish mercury concentrations than fish length (Pickhardt et al. 2002; Sackett et al. 2013). However, acquiring age data can be a lengthy process and commonly requires euthanizing the fish.

Surprisingly, of all the panfish species (bluegill, crappie, and yellow perch) collected, yellow perch had the lowest mercury concentrations. Yellow perch tend to be more piscivorous than bluegill (Knight et al. 1984; www.fishbase.org; last accessed 8/29/16), that should result in

higher mercury concentrations. However, only a few yellow perch were collected from three lakes, and their results based on a limited number of observations should be interpreted cautiously.

Mean depth and watershed area to lake area ratio were positively related to fish mercury concentrations, whereas lake area was negatively related to fish mercury concentrations, suggesting that lake and watershed morphometry can influence mercury cycling in lakes. Fish mercury concentrations often increase with mean depth (Kidd et al. 2012) and have been shown to both increase (Kidd et al. 2012) and decrease (Bodaly et al. 1993) with lake surface area. Inconsistency in these findings further suggests fish mercury concentrations are likely influenced by multiple lake factors. Simple metrics such as lake area or mean depth are a good measure of lake size, but may overlook characteristics associated with lake size, such as lake stratification of oxygen that may be positively related to fish mercury concentrations (Beutel 2016). Anoxic zones tend to have favorable conditions for mercury-fixing bacteria (low pH and low dissolved oxygen; Shao et al. 2012; Garcia et al. 2013). Anoxic zones occur in the deepest parts of some of the study lakes and were hypothesized to explain some of the variation in fish mercury concentrations across lake systems. However, frequency of hypoxic conditions was not found to influence fish mercury concentrations in Iowa lakes.

In contrast, shallow natural lakes tended to have the highest detected fish mercury concentrations. Shallow lakes with agriculturally dominated watersheds are generally known for having poor water quality (Hall et al. 1999). However, eutrophication is often negatively related to fish mercury concentrations due to growth biodilution (Sunda and Huntsman 1998; Pickhardt et al. 2002). Conceptually, biota (phytoplankton, zooplankton, and fishes) production increases in nutrient rich systems, potentially causing a dilution of mercury during trophic transfer, leading

to reduced mercury concentrations in biota at higher trophic levels (Sunda and Huntsman 1998; Pickhardt et al. 2002). Although TSI was not an important predictor of fish mercury concentrations, there still may a relationship between lake eutrophication and fish mercury concentrations. In Iowa, 90% (n = 27) of lakes were considered to be in a eutrophic state (TSI = 50-70; Carlson 1977; Carlson and Simpson 1996), that may have obscured the relationship between eutrophication and fish mercury concentrations.

While ecoregions are not directly influencing fish mercury concentrations, they are defined by and comprised of a suite of landscape and geological factors that can influence fish mercury concentrations (Sackett et al. 2009; Glover et al. 2010). Studies evaluating fishes from North Carolina and South Carolina found similar differences in mercury concentrations across level-III ecoregions (Sackett et al. 2009; Glover et al. 2010). Level-IV ecoregions were used in this study because majority of Iowa is comprised of only one level-III ecoregion (47-Western Corn Belt Plains); yet, differences fish mercury concentrations were still found across ecoregions. Though most ecoregions had similar mercury concentrations, the CIP ecoregion, that included Lake Geode, Lake Miami, Rathbun Reservoir, Red Haw Lake, and Lake Wapello, had the highest fish mercury concentrations. The CIP ecoregion is characterized by irregular topography, relatively higher precipitation, and considerably larger riparian zones comprised of deciduous forest compared to other ecoregions (Griffith et al. 1994; Rowe et al. 2009). The watersheds of these lakes also have relatively high percentages of forest and low percentages of agriculture (Appendix D) that may be contributing to higher fish mercury concentrations.

Watershed size and land use characteristics were important in explaining variation in fish mercury concentrations across Iowa lakes. Similar to previous observations (Rypel 2010; Hayer et al. 2011), fish mercury concentrations increased with watershed size, potentially due to larger

drainage areas and sources of mercury. Within watersheds, wetland area is often positively related to fish mercury concentrations due to increased methymercury production in wetland areas (Rypel 2010; Wentz et al. 2014). However, wetland area was not related to fish mercury concentrations in Iowa lakes. Instead, mercury concentrations were negatively related to agricultural land use in Iowa, potentially due to increased lake productivity and mercury biodilution (Sunda and Huntsman 1998; Pickhardt et al. 2002). Conversely, agricultural land use was positively related to sediment (Kamman et al. 2005) and fish (Sackett et al. 2009) mercury concentrations in other systems. Finally, the percentage of developed land in lake watersheds was also negatively related to fish mercury concentrations in Iowa, even though variation in developed land among watersheds was small (Table 3.2). Thus, even small differences in the percent of developed land within a watershed may affect fish mercury concentrations.

Surprisingly few water chemistry variables were retained in the top model during the first regsubsets procedure. One exception was that pH was positively related to mercury concentrations. However, pH is often negatively related to fish mercury concentrations (e.g., Wren and MacCrimmon 1983; Driscoll et al. 1994; Hakanson 2003). While many variables were compiled for this study, none addressed the trophic structure of the fish populations sampled. Piscivorous fishes generally have higher mercury concentrations than planktivorous fishes due to bioaccumulation. Thus, future work should evaluate the effect of fish trophic positions and their role in determining fish mercury concentrations. Specifically, stable isotopes of $\delta^{15}N$ and $\delta^{13}C$ may explain some of the unexplained variation in fish tissue concentrations in this study.

The predictive two-part model produced from this study serves two general purposes. First, it can be used to describe differences in fish mercury concentrations on many different spatial and biological scales. Second, it can be used with existing information about lakes to

predict which lakes yet to be sampled may contain fish with elevated mercury concentrations. This has important implications for management because it will improve which lakes fish sampling efforts should be directed towards for mercury monitoring. If this model can be validated, the model will have important implications for fish consumption advisories throughout the state, as it will predict both areas of elevated mercury concentrations and areas with low mercury concentrations.

This study provides a comprehensive analysis of factors influencing mercury concentrations in Iowa fishes. This study also serves as evidence to suggest fish mercury concentrations are influenced by a suite of abiotic and biotic factors within and across waterbodies. It is clear that mechanisms driving variation in fish mercury concentrations can vary considerably from region to region. As fish mercury concentrations are highly variable, I suggest a holistic approach to determining factors influencing mercury concentrations.

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Tables and Figures

Table 3.1. Characteristics of 30 Iowa lakes examined in this study, including lake type (CL = constructed lake; NL = natural lake; R = reservoir; SNL = shallow natural lake), ecoregion (CIP = Central Irregular Plains; DSML = Des Moines Lobe; IS = Iowan Surface; LHSRP = Loess Hills and Steeply Rolling Prairies; NILP = Northwest Iowa Loess Prairies; PP = Paleozoic Plateau; SIRLP = Southern Iowa Rolling Loess Prairies), coordinates (Northing and Easting), mean depth (MD, m), lake area (LA, ha), watershed area to lake area ratio (WA:LA ratio), and years since construction (YSC) for reservoirs and constructed lakes. Lake locations are provided in Figure 3.2.

Waterbody	ID	Lake type	Ecoregion	Northing	Easting	MD	LA	WA:LA	YSC
Ahquabi	1	CL	SIRLP	4571146	450319.3	3.0	47.0	14.9	80
Anita	2	CL	SIRLP	4587776	351183.9	3.8	71.9	13.0	48
Beeds	3	CL	DSML	4735292	480055.9	2.6	39.7	207.7	81
Big Creek	4	CL	DSML	4629479	438321.2	5.5	349.6	54.2	43
Briggs Woods	5	CL	DSML	4698625	434799.4	3.7	24.0	120.7	47
Clear	6	NL	DSML	4775663	468224.0	2.9	1484.8	2.6	0
Coralville	7	R	SIRLP	4620484	622310.0	4.3	2136.7	376.4	57
Crystal	8	SNL	DSML	4786527	435751.7	1.4	106.9	7.5	0
Geode	9	CL	CIP	4519879	636088.0	7.2	76.8	53.4	65
Hendricks	10	CL	IS	4802512	536796.8	2.4	18.1	26.0	55
Lake of the Hills	11	CL	SIRLP	4599252	693799.0	3.0	21.7	30.7	43
Little Wall	12	SNL	DSML	4679896	447509.2	1.6	99.5	0.8	0
Miami	13	CL	CIP	4551707	512952.9	3.0	55.9	28.0	49
Mormon Trail	14	CL	SIRLP	4566934	363054.2	4.2	13.7	11.4	48
North Twin	15	NL	DSML	4704862	366058.1	3.0	185.1	4.6	0
Okoboji	16	NL	DSML	4805142	328394.9	8.5	2322.8	3.3	0
Pleasant Creek	17	CL	IS	4664126	598199.6	5.0	169.3	4.9	39
Rathbun	18	R	CIP	4521817	507933.7	6.3	4379.5	10.9	46
Red Haw	19	CL	CIP	4538562	477089.4	4.4	29.4	13.0	77
Red Rock	20	R	DSML	4581032	500001.3	5.5	6171.5	517.0	47
Saylorville	21	R	DSML	4618589	442684.7	4.6	2407.9	625.0	38
Silver	22	NL	DSML	4813089	310997.3	1.9	432.4	14.2	0
Spirit	23	NL	DSML	4812894	329967.0	5.2	2174.5	3.2	0
Storm	24	NL	NILP	4720590	320724.0	2.3	1271.5	4.7	0
Three Mile	25	CL	SIRLP	4547597	397910.9	5.0	322.5	27.5	20
Twelve Mile	26	CL	SIRLP	4545747	394545.9	4.6	257.4	22.0	32
Viking	27	CL	LHSRP	4538071	329002.1	4.7	58.4	14.0	56
Volga	28	CL	PP	4750319	600326.8	3.2	53.6	45.0	36
Wapello	29	CL	CIP	4518715	535775.6	3.9	113.5	17.0	80
Yellow Smoke	30	CL	LHSRP	4655290	307776.4	3.3	16.1	37.6	36

Table 3.2. Species-specific sample size (N), proportion of N below the detection limit (0.05 mg/kg), proportion of samples by sex (Female, Male, Unknown), mean detected mercury concentration (mg/kg), maximum mercury concentration (mg/kg), mean total length (TL, mm), minimum and maximum fish length (mm), minimum, maximum, and mean age, and the number of waterbodies each species was sampled from. R^2 and *P*-values refer to simple linear regressions between log-transformed mercury concentrations and total length (mm) and age by species. NOP = northern pike, MUE = muskellunge, BLG = bluegill, LMB = largemouth bass, YEP = yellow perch, WHC = white crappie, BLC = black crappie, CRP = black and white crappie combined, WAE = walleye. Means sharing a common letter are not significantly different (ANOVA; $P > 0.05$).

Attribute	Species								
Species code	NOP	MUE	BLG	LMB	YEP*	WHC	BLC	CRP	WAE
N/% < detect	45/9%	30/3%	275/87%	502/23%	44/93%	112/81%	203/63%	315/69%	248/26%
Female (%)	71	40	43	24	43	41	36	38	39
Male (%)	24	50	35	33	41	47	43	45	55
Unknown (%)	4	10	23	43	16	12	20	17	6
Mean detect Hg	0.23 [a,c]	0.35 [a,c]	0.09 [b]	0.24 [a]	0.08	0.11	0.1	0.1 [b]	0.22 [c]
Max. Hg	0.65	2.52	0.27	0.82	0.10	0.24	0.25	0.25	0.86
TL R^2/*P*-value	0.11/0.03	0.52/<0.01	0.24/<0.01	0.40/<0.01	N/A	N/A	N/A	<0.01/0.76	0.24/<0.01
Age R^2/*P*-value	0.08/0.08	0.56/<0.01	0.21/<0.01	0.35/<0.01	N/A	N/A	N/A	0.32/<0.01	0.49/<0.01
Mean TL	686	940	151	344	206	239	219	226	505
Min.-Max. TL	370-945	723-1204	78-259	190-539	127-281	127-394	116-335	116-394	256-747
Min.-Max. Age	2-7	3-14	1-10	1-15	0-6	1-10	1-13	1-13	0-16
Mean Age	4.4	7.4	3.0	5.0	2.7	3.4	3.5	3.5	6.0
# of lakes	3	3	26	26	4	13	22	26	13

* = Omitted from predictive models due to low sample size of detected mercury concentrations.

Table 3.3. Mean fish mercury concentrations (Hg; mg/kg) by species and waterbody. NOP = northern pike, MUE = muskellunge, BLG = bluegill, LMB = largemouth bass, CRP = black and white crappie combined, WAE = walleye. Undct = all observations were undetected mercury concentrations. Note: undetected mercury concentrations were assigned a value of 0.025 mg/kg.

Waterbody	NOP	MUE	BLG	LMB	CRP	WAE	All Species
Ahquabi	-	-	Undct	0.14	0.04	-	0.07
Anita	-	-	Undct	0.16	0.06	-	0.08
Beeds	-	-	Undct	0.06	Undct	-	0.04
Big Creek	-	-	0.04	0.10	0.05	0.16	0.09
Briggs Woods	-	-	Undct	0.13	Undct	-	0.07
Clear	-	0.09	-	-	Undct	0.10	0.07
Coralville	-	-	Undct	0.05	Undct	0.11	0.05
Crystal	0.10	-	Undct	0.19	Undct	-	0.08
Geode	-	-	0.09	0.29	-	-	0.19
Hendricks	-	-	-	0.18	-	-	0.18
Lake of the Hills		-	Undct	0.10	0.05	-	0.06
Little Wall	-	-	Undct	0.37	0.14	-	0.18
Miami	-	-	Undct	0.11	Undct	-	0.05
Mormon Trail	-	-	0.04	0.17	0.09	-	0.09
North Twin	-	-	Undct	-	Undct	0.04	0.03
Okoboji	0.27	0.59	0.05	0.22	0.06	0.24	0.23
Pleasant Creek	-	-	Undct	0.16	Undct	0.10	0.08
Rathbun	-	-	Undct	0.22	0.05	0.26	0.21
Red Haw	-	-	Undct	0.19	-	-	0.18
Red Rock	-	-	Undct	0.10	0.04	0.03	0.05
Saylorville	-	-	Undct	0.16	0.07	-	0.09
Silver	-	-	-	-	-	0.06	0.06
Spirit	0.21	0.18	0.05	0.19	0.07	0.23	0.17
Storm	-	-	-	-	0.03	0.08	0.06
Three Mile	-	-	Undct	0.16	0.05	0.27	0.11
Twelve Mile	-	-	0.06	0.19	0.05	0.13	0.16
Viking	-	-	Undct	0.24	0.05	-	0.10
Volga	-	-	Undct	0.05	0.04	-	0.04
Wapello	-	-	0.05	0.50	0.14	-	0.26
Yellow Smoke	-	-	Undct	0.20	0.25	-	0.14

Table 3.4. Mean detected fish mercury concentrations (Hg; mg/kg) by species and waterbody. NOP = northern pike, MUE = muskellunge, BLG = bluegill, LMB = largemouth bass, CRP = black and white crappie combined, WAE = walleye.

Waterbody	NOP	MUE	BLG	LMB	CRP	WAE	All Species
Ahquabi	-	-	-	0.21	0.08	-	0.18
Anita	-	-	-	0.16	0.11	-	0.15
Beeds	-	-	-	0.23	-	-	0.23
Big Creek	-	-	0.13	0.16	0.09	0.16	0.14
Briggs Woods	-	-	-	0.24	-	-	0.24
Clear	-	0.09	-	-	-	0.16	0.13
Coralville	-	-	-	0.15	-	0.13	0.13
Crystal	0.20	-	-	0.22	-	-	0.21
Geode	-	-	0.14	0.32	-	-	0.25
Hendricks	-	-	-	0.18	-	-	0.18
Lake of the Hills	-	-	-	0.18	0.11	-	0.15
Little Wall	-	-	-	0.41	0.22	-	0.36
Miami	-	-	-	0.37	-	-	0.37
Mormon Trail	-	-	0.07	0.26	0.09	-	0.13
North Twin	-	-	-	-	-	0.19	0.19
Okoboji	0.27	0.59	0.14	0.27	0.09	0.25	0.29
Pleasant Creek	-	-	-	0.23	-	0.14	0.20
Rathbun	-	-	-	0.33	0.20	0.28	0.29
Red Haw	-	-	-	0.22	-	-	0.22
Red Rock	-	-	-	0.20	0.07	0.05	0.11
Saylorville	-	-	-	0.20	0.11	-	0.17
Silver	-	-	-	-	-	0.06	0.06
Spirit	0.21	0.18	0.06	0.19	0.09	0.24	0.19
Storm	-	-	-	-	-	0.15	0.15
Three Mile	-	-	-	0.19	0.13	0.34	0.23
Twelve Mile	-	-	0.15	0.23	0.07	0.25	0.21
Viking	-	-	-	0.24	0.11	-	0.21
Volga	-	-	-	0.17	0.09	-	0.12
Wapello	-	-	0.06	0.50	0.14	-	0.26
Yellow Smoke	-	-	-	0.20	0.25	-	0.20

Table 3.5. Top ten multiple regression models developed to predict fish mercury concentrations using fish-level variables (see Methods) ordered by Akaike's information criterion (AIC_c) using regression subset selection procedure. K = the number of parameters in the model (includes Waterbody), and ΔAIC_c = the distance of each model from the best AIC_c model, and w_i = the model weight (a measure of relative strength). Each model was produced from 770 observations.

Model	K	AIC_c	ΔAIC_c	w_i
Species, Sex, JD, SW, Age, pH, DOC, Species*Sex, Species*Age, Species*TL, Sex*TL	50	-951.06	0.00	0.27
Species, Sex, JD, SW, Age, pH, Alkalinity, DOC, Species*Sex, Species*Age, Species*TL, Sex*TL	51	-950.93	0.14	0.25
Species, Sex, JD, SW, Age, DOC, Species*Sex, Species*Age, Species*TL, Sex*TL	46	-950.02	1.04	0.16
Species, Sex, JD, SW, Age, Turb, pH, Alkalinity, DOC, Species*Sex, Species*Age, Species*TL, Sex*TL	54	-949.66	1.40	0.13
Species, JD, TL, SW, Age, DOC, Species*Sex, Species*Age, Species*TL, Sex*TL	45	-948.72	2.35	0.08
Species, TL, SW, Age, DOC, Species*Sex, Species*Age, Species*TL, Sex*TL	43	-948.23	2.84	0.06
Species, TL, SW, Age, DOC, Species*Sex, Species*Age, Species*TL	41	-947.17	3.89	0.04
Species, TL, SW, Age, Species*Age, Species*TL	40	-943.97	7.09	0.01
Species, Sex, JD, TL, SW, Age, Turb, pH, Alkalinity, DOC, Species*Sex, Species*Age, Species*TL, Sex*TL	59	-941.74	9.33	0.00
Species, Sex, JD, TL, SW, Age, Turb, TSI, pH, Alkalinity, DOC, Species*Sex, Species*Age, Species*TL, Sex*TL	63	-935.23	15.84	0.00

Table 3.6. Mean detected fish mercury concentrations (Hg; mg/kg), standard deviation (SD), and sample size (n) by species and sex. NOP = northern pike, MUE = muskellunge, BLG = bluegill, LMB = largemouth bass, CRP = black and white crappie combined, WAE = walleye. Means sharing a common letter within the all species category are not significantly different (ANOVA; $P > 0.05$).

Sex	Metric	NOP	MUE	BLG	LMB	CRP	WAE	All species
Female	Mean Hg	0.26	0.49	0.10	0.27	0.11	0.23	0.23 [a,b]
	SD	0.12	0.70	0.06	0.15	0.05	0.17	0.21
	n	28	12	16	86	47	82	271
Male	Mean Hg	0.18	0.28	0.10	0.22	0.09	0.21	0.20 [a]
	SD	0.05	0.21	0.04	0.11	0.05	0.14	0.13
	n	11	14	16	130	37	96	304
Unknown	Mean Hg	0.16	0.11	0.06	0.23	0.11	0.17	0.22 [b]
	SD	0.08	0.01	0.02	0.13	0.05	0.11	0.13
	n	2	3	3	170	13	5	196

Table 3.7. Parameter estimates (± 95% confidence intervals; C.I.) for all variables and interactions retained in the final predictive multiple regression model.

Variable	Parameter estimate	± 95% C.I.		Variable	Parameter estimate	± 95% C.I.	
Intercept	-9.13	-12.28	-5.99	WA.LA	0.48	0.27	0.68
LMB	0.37	-0.26	1.00	TL	0.003	-0.001	0.01
MUE	-0.07	-1.69	1.55	SexMale*LMB	-0.01	-0.32	0.30
NOP	2.33	1.41	3.25	SexMale *MUE	-0.03	-0.74	0.67
CRP	1.16	0.47	1.84	SexMale *NOP	-0.31	-0.83	0.22
WAE	1.41	0.68	2.14	SexMale *CRP	-0.19	-0.50	0.12
SexMale	0.10	-0.20	0.40	SexMale *WAE	-0.09	-0.49	0.32
SexUnk	-0.17	-0.62	0.29	SexUnk*LMB	-0.19	-0.73	0.34
JD	-0.001	-0.002	-0.0007	SexUnk *MUE	-1.42	-2.57	-0.27
SW	-0.28	-0.38	-0.17	SexUnk *NOP	-1.35	-2.42	-0.28
Age	0.14	0.04	0.25	SexUnk *CRP	-0.26	-0.78	0.27
pH	0.68	0.41	0.95	SexUnk *WAE	-0.80	-1.53	-0.07
DOC	-0.01	-0.04	0.02	LMB*Age	-0.08	-0.18	0.03
LA	-0.0001	-0.0002	-0.00002	MUE*Age	0.04	-0.10	0.18
MD	0.17	0.13	0.22	NOP*Age	-0.09	-0.29	0.10
logperWater	0.42	0.18	0.66	CRP*Age	-0.04	-0.15	0.08
logperFor	-0.11	-0.22	-0.01	WAE*Age	0.01	-0.10	0.12
logperGrass	-0.29	-0.55	-0.04	TL*LMB	0.001	-0.004	0.01
logperAg	-0.44	-0.59	-0.29	TL*MUE	-0.002	-0.01	0.003
logperDev	-0.11	-0.16	-0.05	TL*NOP	-0.003	-0.01	0.002
LakeTypeNL	-0.01	-0.26	0.25	TL*CRP	-0.003	-0.01	0.002
LakeTypeR	-0.001	-0.31	0.31	TL*WAE	-0.003	-0.01	0.001
LakeTypeSNL	0.69	0.34	1.04	TL*SexMale	-0.0002	-0.0010	0.0006
EcoregionDSML	-0.50	-0.81	-0.20	TL*SexUnk	0.001	0.0003	0.003
EcoregionIS	-0.18	-0.38	0.02				
EcoregionLHSRP	0.19	-0.04	0.42				
EcoregionNILP	-0.63	-1.12	-0.13				
EcoregionPP	-0.16	-0.53	0.22				
EcoregionSIRLP	-0.14	-0.36	0.07				

Table 3.8. Top ten multiple regression models developed to predict fish mercury concentrations using lake-level variables (see Methods) ordered by Akaike's information criterion (AIC_c) using regression subset selection procedure. K = the number of parameters in the model (includes fish-level variables species, sex, age, sample weight, and alkalinity), ΔAIC_c = the distance of each model from the best AIC_c model, and w_i = the model weight (a measure of relative strength). Each model was produced from 770 observations.

Model	K	AIC_c	ΔAIC_c	w_i
LA, MD, logperWater, logperFor, logperGrass, logperAg, logperDev, LakeType, Ecoregion, WA.LA	48	937.97	0.00	0.33
Freq.hyp, Northing, LA, MD, logperWater, logperWet, logperAg, logperDev, LakeType, Ecoregion, WA.LA	46	937.12	0.86	0.21
Easting, LA, MD, logperWater, logperWet, logperFor, logperGrass, logperAg, logperDev, LakeType, Ecoregion, WA.LA	50	936.83	1.14	0.19
Freq.hyp, Northing, LA, MD, logperWet, logperAg, logperDev, LakeType, Ecoregion, WA.LA	45	936.70	1.28	0.17
Freq.hyp, LA, MD, logperWet, logperAg, logperDev, LakeType, Ecoregion, WA.LA	44	934.21	3.76	0.05
Northing, LA, MD, WA, logperWater, logperFor, logperGrass, logperAg, logperDev, LakeType, Ecoregion, WA.LA	53	932.21	5.76	0.02
Northing, Easting, LA, MD, WA, logperWater, logperFor, logperGrass, logperAg, logperDev, LakeType, Ecoregion, WA.LA	54	931.17	6.80	0.01
LA, MD, logperWet, logperAg, logperDev, LakeType, Ecoregion, WA.LA	43	930.55	7.42	0.01
Freq.hyp, Easting, LA, MD, WA, logperWater, logperWet, logperFor, logperGrass, logperAg, logperDev, LakeType, Ecoregion, WA.LA	55	930.32	7.66	0.01
Freq.hyp, Northing, Easting, LA, MD, WA, logperWater, logperWet, logperFor, logperGrass, logperAg, logperDev, LakeType, Ecoregion, WA.LA	56	928.47	9.50	0.00

Table 3.9. Mean detected fish mercury concentrations (Hg; mg/kg), standard deviation (SD), and sample size (n) by species and ecoregion. CIP = Central Irregular Plains; DSML = Des Moines Lobe; IS = Iowan Surface; LHSRP = Loess Hills and Steeply Rolling Prairies; NILP = Northwest Iowa Loess Prairies; PP = Paleozoic Plateau; SIRLP = Southern Iowa Rolling Loess Prairies. NOP = northern pike, MUE = muskellunge, BLG = bluegill, LMB = largemouth bass, CRP = black and white crappie combined, WAE = walleye. Means sharing a common letter within the all species category are not significantly different (ANOVA; $P > 0.05$).

Ecoregion	Metric	NOP	MUE	BLG	LMB	CRP	WAE	All Species
CIP	Mean Hg	-	-	0.09	0.26	0.15	0.28	0.24 [a]
	SD	-	-	0.06	0.15	0.03	0.14	0.15
	n	0	0	16	154	11	46	227
DSML	Mean Hg	0.23	0.35	0.09	0.24	0.10	0.19	0.21 [b]
	SD	0.11	0.48	0.04	0.14	0.05	0.16	0.21
	n	41	29	11	62	36	97	276
IS	Mean Hg	-	-	-	0.20	-	0.14	0.19 [a,b]
	SD	-	-	-	0.08	-	0.02	0.08
	n	0	0	0	15	0	3	18
LHSRP	Mean Hg	-	-	-	0.22	0.14	-	0.20 [a,b]
	SD	-	-	-	0.16	0.10	-	0.15
	n	0	0	0	23	5	0	28
NILP	Mean Hg	-	-	-	-	-	0.15	0.15 [b]
	SD	-	-	-	-	-	0.10	0.10
	n	0	0	0	0	0	11	11
PP	Mean Hg	-	-	-	0.17	0.09	-	0.12 [a,b]
	SD	-	-	-	0.08	0.05	-	0.07
	n	0	0	0	2	3	0	5
SIRLP	Mean Hg	-	-	0.10	0.22	0.09	0.23	0.19 [b]
	SD	-	-	0.04	0.09	0.03	0.16	0.11
	n	0	0	8	130	42	26	206

Table 3.10. Mean detected fish mercury concentrations (Hg; mg/kg), standard deviation (SD), and sample size (n) by species and lake type. CL = constructed lake; NL = natural lake; R = reservoir; SNL = shallow natural lake. NOP = northern pike, MUE = muskellunge, BLG = bluegill, LMB = largemouth bass, CRP = black and white crappie combined, WAE = walleye. Means sharing a common letter within the all species category are not significantly different (ANOVA; $P > 0.05$).

Lake Type	Metric	NOP	MUE	BLG	LMB	CRP	WAE	All species
CL	Mean Hg	-	-	0.10	0.23	0.10	0.23	0.21 [a]
	SD	-	-	0.06	0.13	0.04	0.15	0.13
	n	0	0	25	330	64	29	448
NL	Mean Hg	0.23	0.35	0.09	0.23	0.09	0.20	0.21 [a]
	SD	0.11	0.48	0.04	0.15	0.03	0.16	0.23
	n	38	29	10	22	20	93	212
R	Mean Hg	-	-	-	0.23	0.10	0.24	0.22 [a]
	SD	-	-	-	0.13	0.06	0.14	0.14
	n	0	0	0	20	10	61	91
SNL	Mean Hg	0.20	-	-	0.33	0.22	-	0.29 [b]
	SD	0.03	-	-	0.16	0.02	-	0.15
	n	3	0	0	14	3	0	20

Table 3.11. Summary of predictions made by the logistic regression model. Undetected refers to predictions with a probability of detection <50%. Predicted refers to predictions with a probability of detection ≥50%.

Observed	Predicted		
	Undetected	Detected	% Correct
Undetected	561	85	87%
Detected	72	697	91%
% Correct	89%	89%	Overall 89%

Table 3.12. Parameter estimates (± 95% confidence intervals; C.I.) for all variables and interactions retained in the logistic regression model.

Variable	Parameter estimate	± 95% C.I.		Variable	Parameter estimate	± 95% C.I.	
Intercept	-4.49	-5.88	-3.09	WA.LA	0.32	0.21	0.44
LMB	0.17	-0.10	0.45	TL	-0.0001	-0.002	0.002
MUE	1.79	0.39	3.18	SexMale*LMB	-0.06	-0.22	0.10
NOP	0.88	0.17	1.60	SexMale *MUE	-0.46	-0.99	0.08
CRP	-0.13	-0.42	0.15	SexMale *NOP	-0.03	-0.43	0.38
WAE	-0.10	-0.50	0.30	SexMale *CRP	-0.08	-0.21	0.05
SexMale	-0.05	-0.18	0.07	SexMale *WAE	-0.08	-0.33	0.18
SexUnk	-0.02	-0.16	0.13	SexUnk*LMB	0.01	-0.21	0.23
JD	0.0001	-0.0003	0.0005	SexUnk *MUE	-0.18	-0.95	0.59
SW	0.54	0.46	0.62	SexUnk *NOP	-0.47	-1.21	0.28
Age	0.05	0.01	0.09	SexUnk *CRP	-0.04	-0.21	0.13
pH	0.30	0.17	0.42	SexUnk *WAE	-0.28	-0.62	0.06
DOC	0.01	-0.01	0.03	LMB*Age	-0.002	-0.05	0.04
LA	-0.00000001	-0.00000001	-0.000000007	MUE*Age	-0.03	-0.13	0.07
MD	0.15	0.12	0.17	NOP*Age	-0.18	-0.33	-0.03
logperWater	0.24	0.10	0.38	CRP*Age	0.04	-0.003	0.09
logperFor	-0.03	-0.08	0.03	WAE*Age	-0.04	-0.09	0.001
logperGrass	0.17	0.07	0.27	TL*LMB	0.001	-0.001	0.003
logperAg	-0.20	-0.29	-0.11	TL*MUE	-0.001	-0.003	0.001
logperDev	-0.03	-0.07	0.01	TL*NOP	0.001	-0.001	0.003
LakeTypeNL	0.45	0.29	0.60	TL*CRP	0.001	-0.001	0.003
LakeTypeR	-0.04	-0.21	0.13	TL*WAE	0.002	-0.0002	0.003
LakeTypeSNL	0.51	0.30	0.72	TL*SexMale	0.0005	-0.0001	0.001
EcoregionDSML	-0.01	-0.18	0.17	TL*SexUnk	0.0004	-0.0004	0.001
EcoregionIS	0.06	-0.07	0.18				
EcoregionLHSRP	-0.12	-0.25	0.01				
EcoregionNILP	0.13	-0.13	0.40				
EcoregionPP	-0.06	-0.19	0.08				
EcoregionSIRLP	-0.12	-0.23	-0.01				

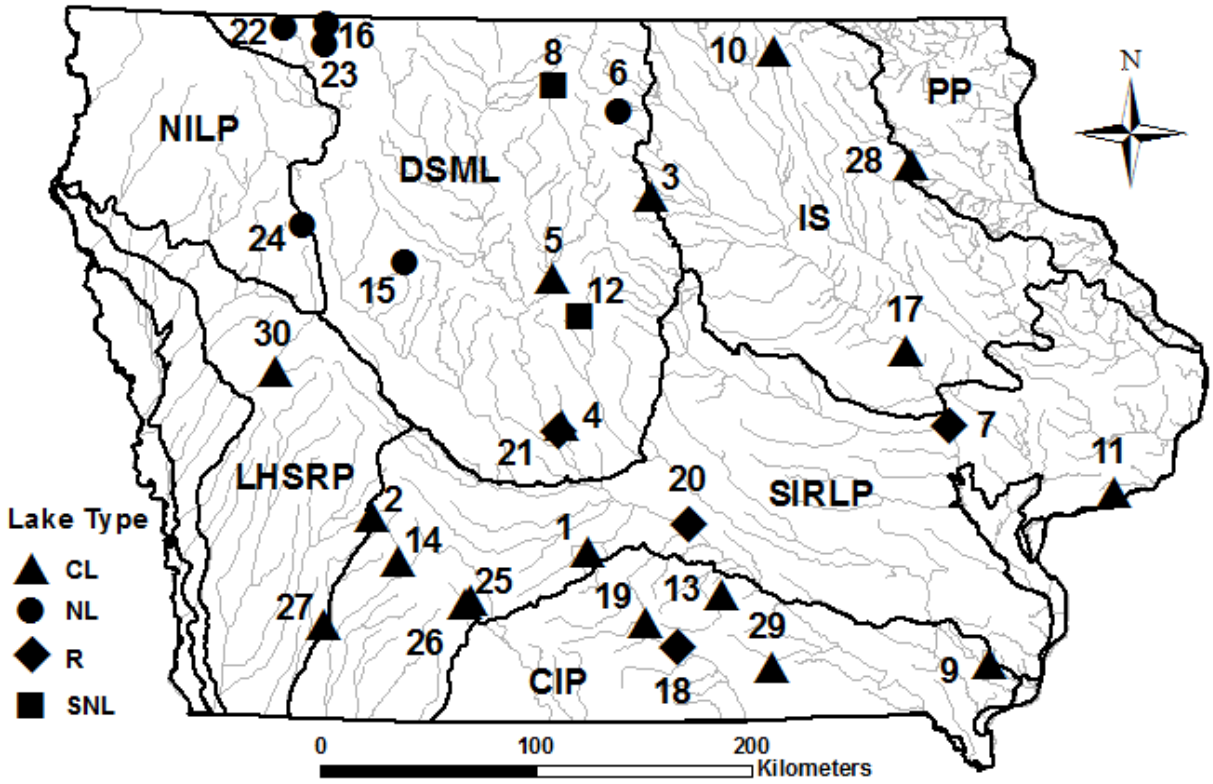

Figure 3.1. Fish sampling locations (black symbols) located throughout Iowa separated by level-4 ecoregions. Numbers next to symbols refer to a lake identification number (Table 3.1). Lake types: CL = constructed lake; NL = natural lake; R = reservoir; SNL = shallow natural lake. Ecoregions: CIP = Central Irregular Plains; DSML = Des Moines Lobe; IS = Iowan Surface; LHSRP = Loess Hills and Steeply Rolling Prairies; NILP = Northwest Iowa Loess Prairies; PP = Paleozoic Plateau; SIRLP = Southern Iowa Rolling Loess Prairies.

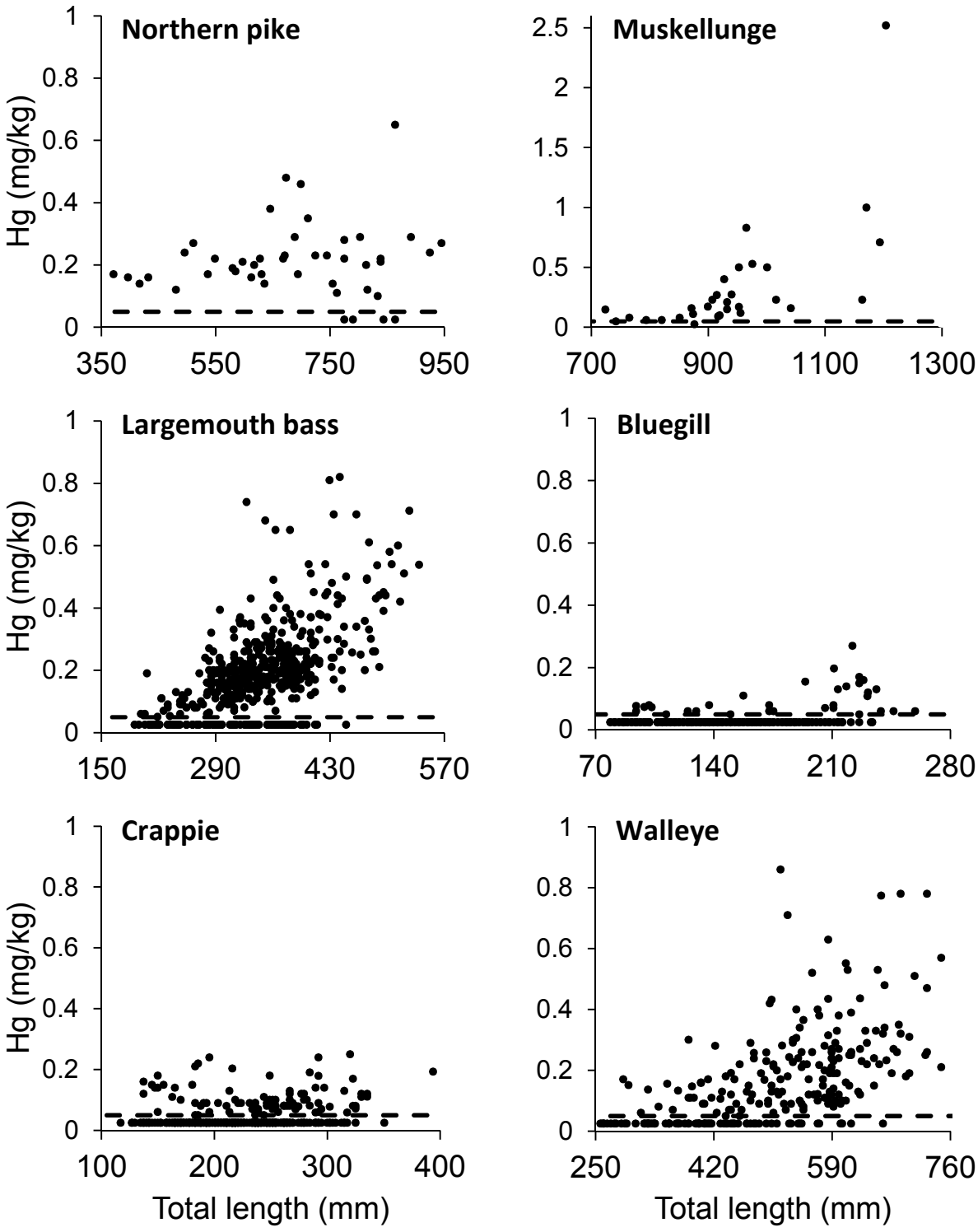

Figure 3.2. Northern pike (n = 45), muskellunge (n = 30), largemouth bass (n = 502), bluegill (n = 275), crappie (n = 315), and walleye (n = 248) mercury concentrations (Hg; mg/kg) versus total length (mm). Dashed line represents the detection limit (0.05). Note: muskellunge have a different y-axis scale compared to the other five species. See appendix K for total length in inches.

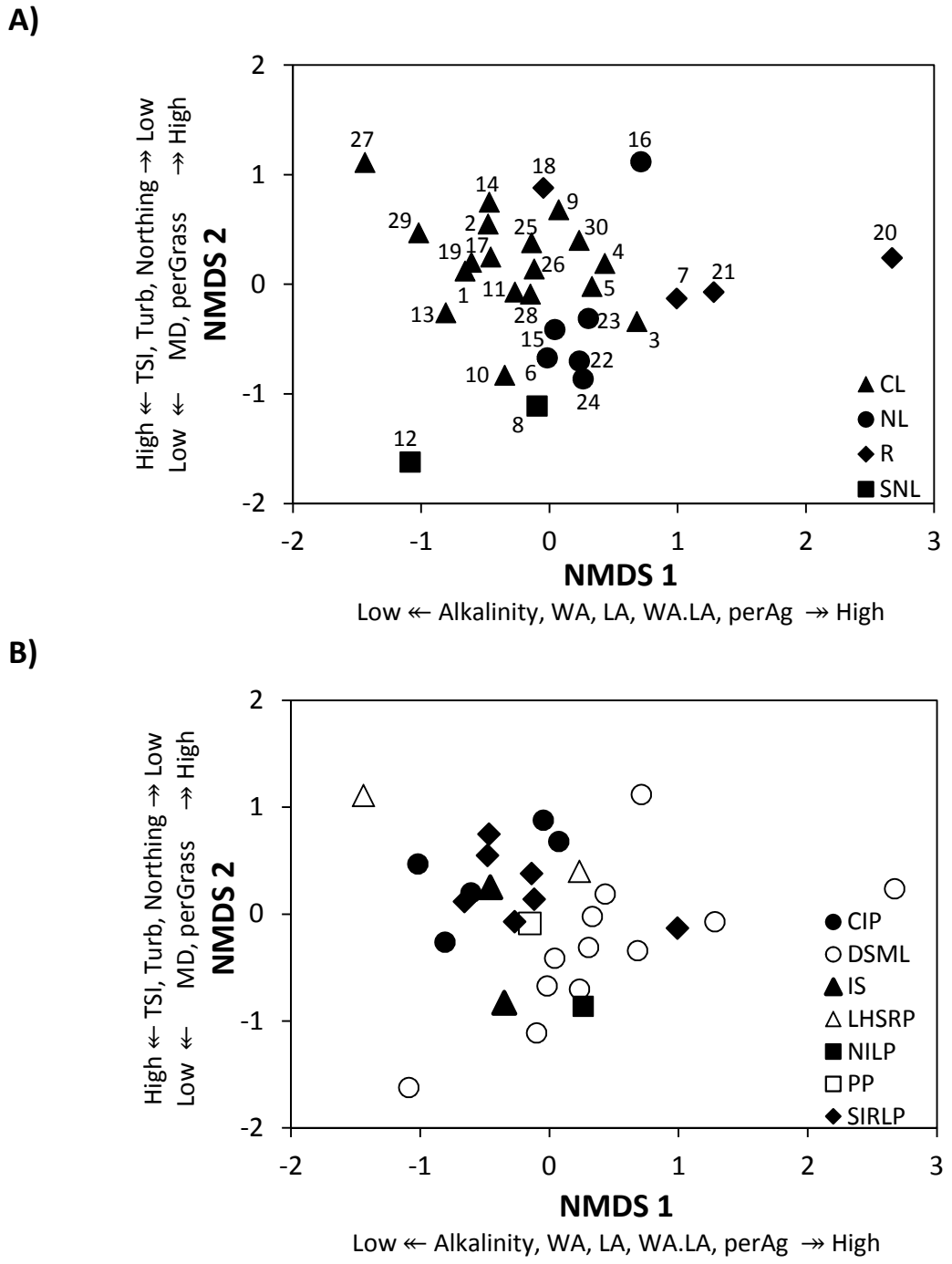

Figure 3.3. NMDS ordination of 30 Iowa lakes based on 17 environmental variables. Lakes are coded by lake type (A; CL = constructed lake; NL = natural lake; R = reservoir; SNL = shallow natural lake) and ecoregion (B; CIP = Central Irregular Plains; DSML = Des Moines Lobe; IS = Iowan Surface; LHSRP = Loess Hills and Steeply Rolling Prairies; NILP = Northwest Iowa Loess Prairies; PP = Paleozoic Plateau; SIRLP = Southern Iowa Rolling Loess Prairies). Numbers (A) identify waterbodies listed in Table 3.1. See Methods or Appendix B for variable abbreviations. Only variable vectors that were significantly correlated with NMDS axes scores are labeled on the axes ($r \geq 0.50$, $P < 0.05$).

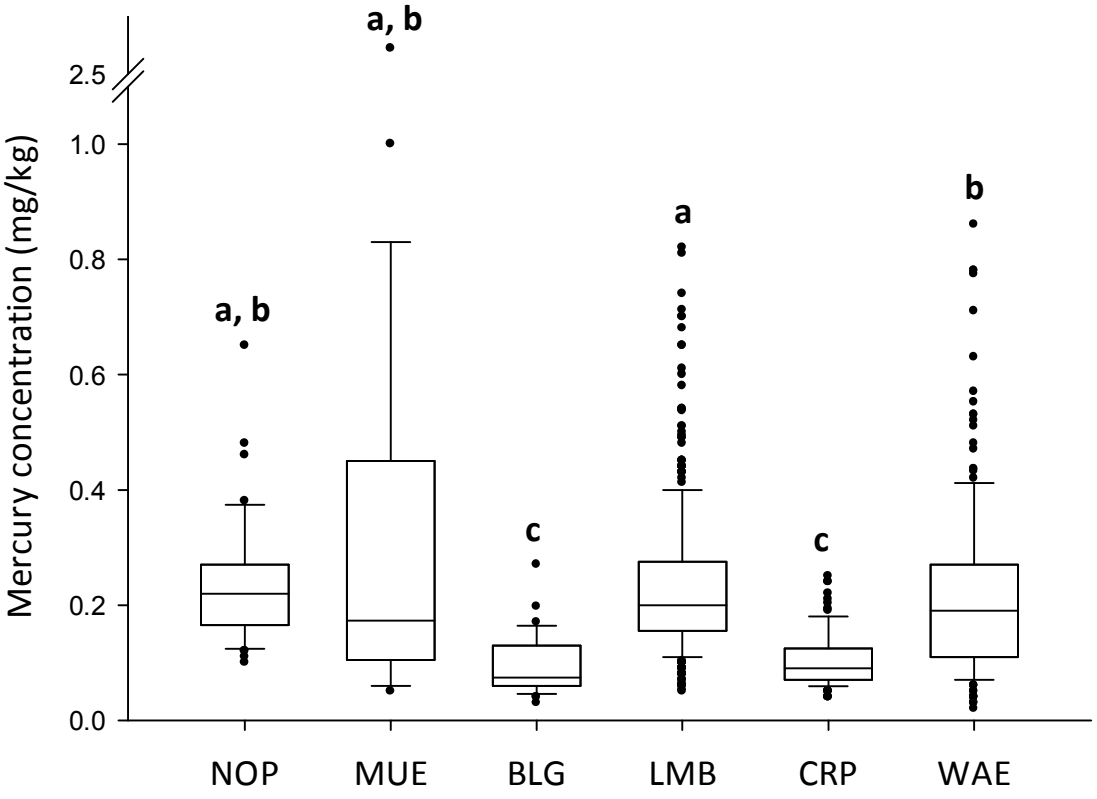

Figure 3.4. Box plots of detected fish mercury concentrations (mg/kg) by species (NOP = northern pike, MUE = muskellunge, BLG = bluegill, LMB = largemouth bass, CRP = black and white crappie combined, WAE = walleye). The line within the box represents the median value. Dimensions of the box represent the 25th and 75th percentiles. Error bars represent the 10th and 90th percentiles. Individual points are outliers. Boxes sharing a common letter are not significantly different (ANOVA; $P > 0.05$).

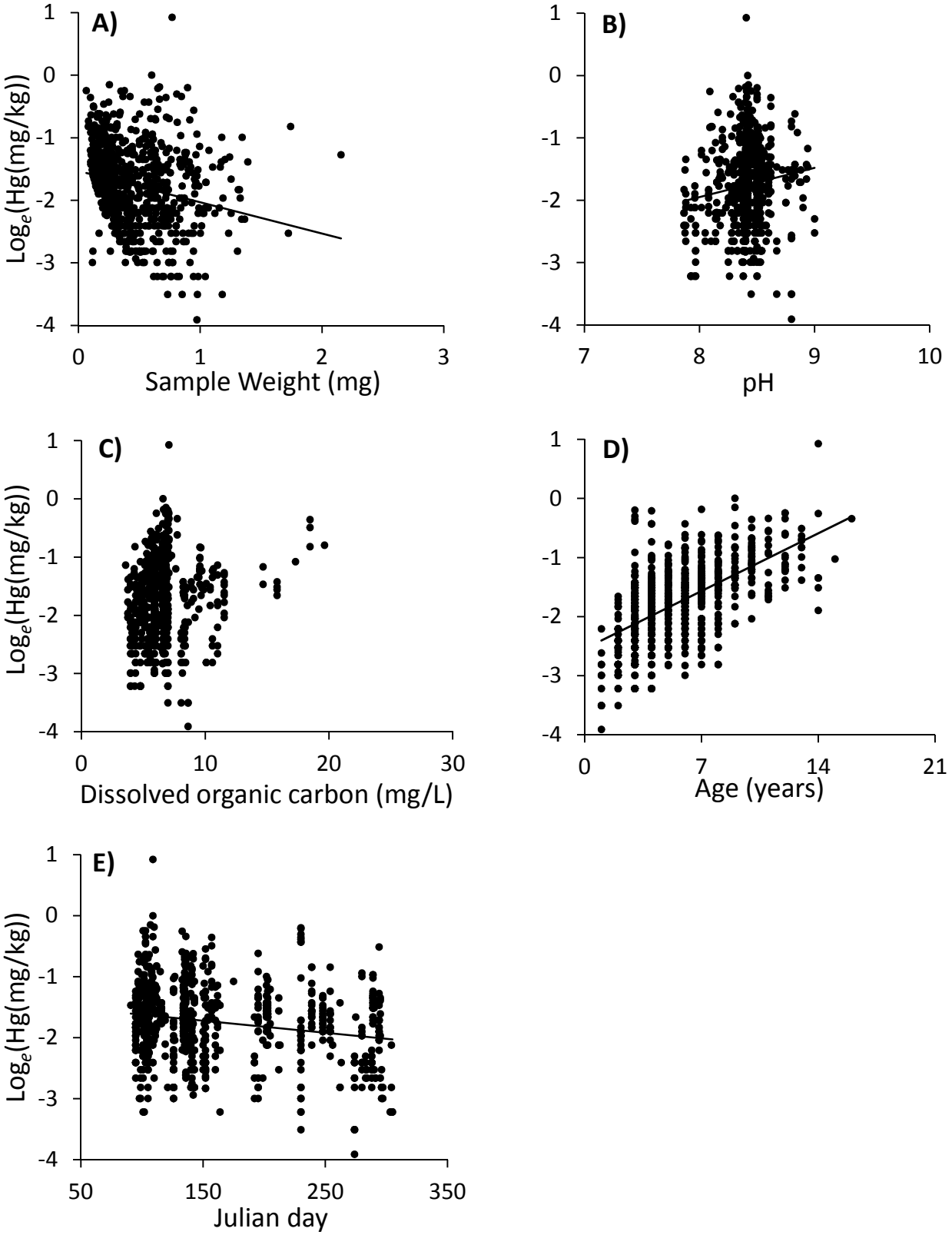

Figure 3.5. Log-transformed detected fish mercury concentrations (mg/kg) plotted versus sample weight (A; mg), pH (B; mg/kg), dissolved organic carbon (C; mg/L), fish age (D; years), and Julian day (E).

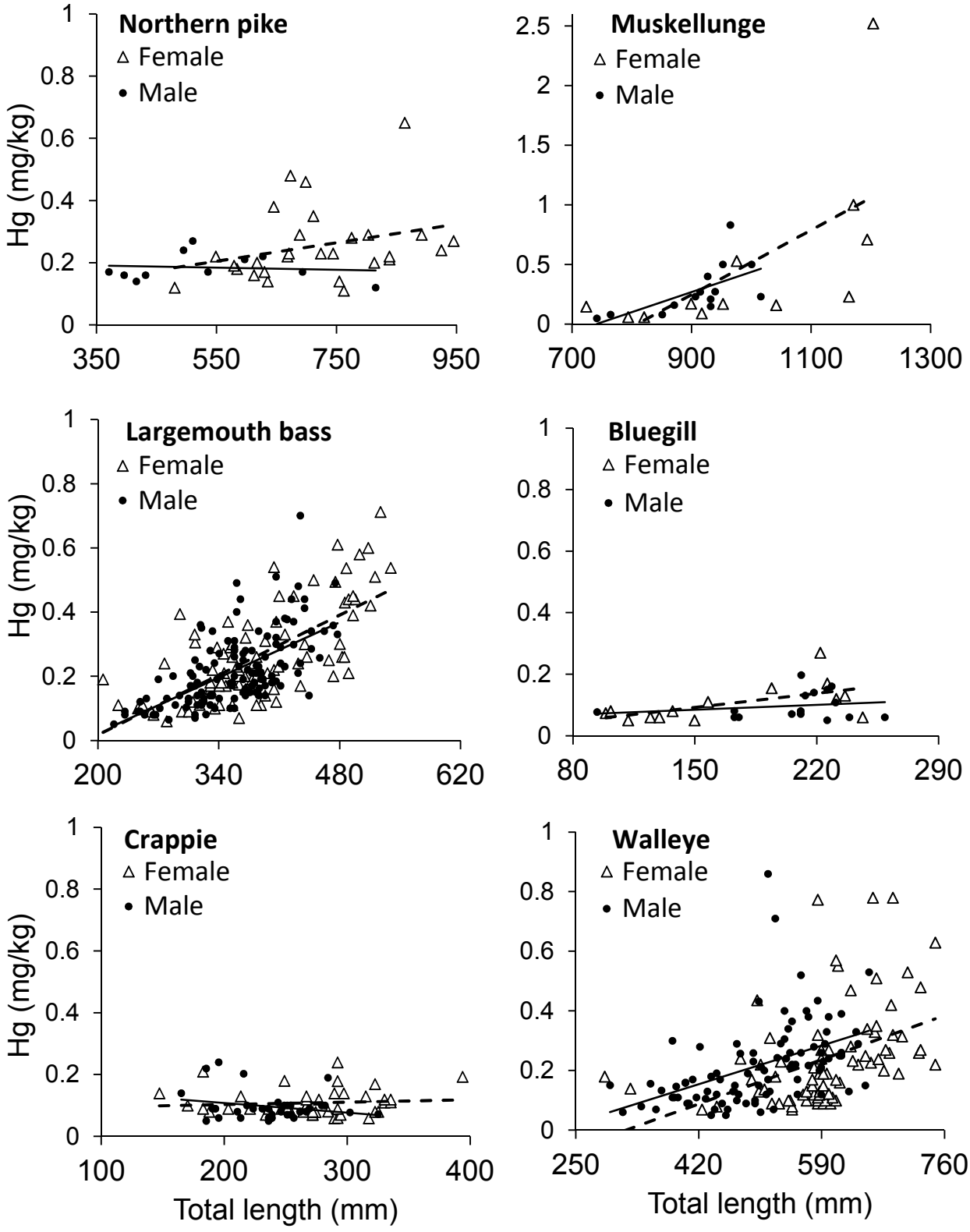

Figure 3.6. Northern pike, muskellunge, largemouth bass, bluegill, crappie, and walleye detected mercury concentrations (mg/kg) versus length (mm) by sex (female =Δ ; male = ●). Dashed line is the regression line for females; solid line is the regression line for males.

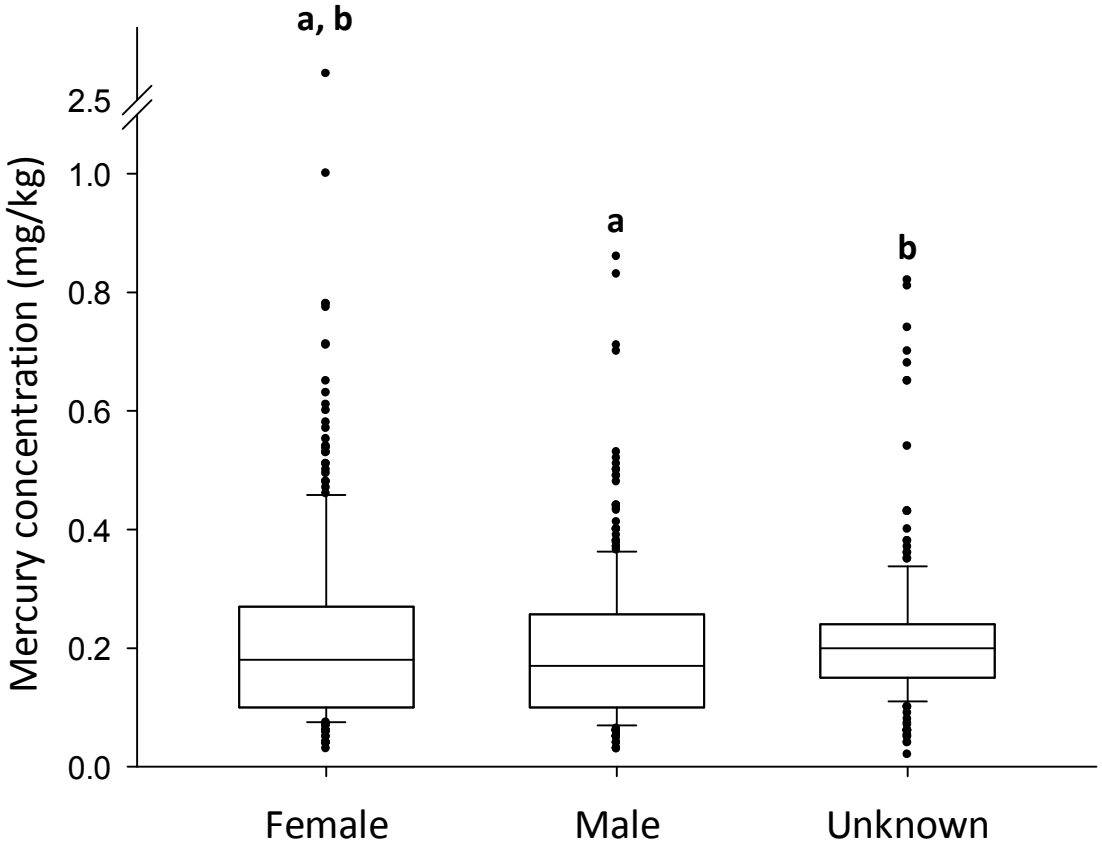

Figure 3.7. Box plots of detected fish mercury concentrations (mg/kg) by sex. The line within the box represents the median value. Dimensions of the box represent the 25th and 75th percentiles. Error bars represent the 10th and 90th percentiles. Individual points are outliers. Boxes sharing a common letter are not significantly different (ANOVA; $P > 0.05$).

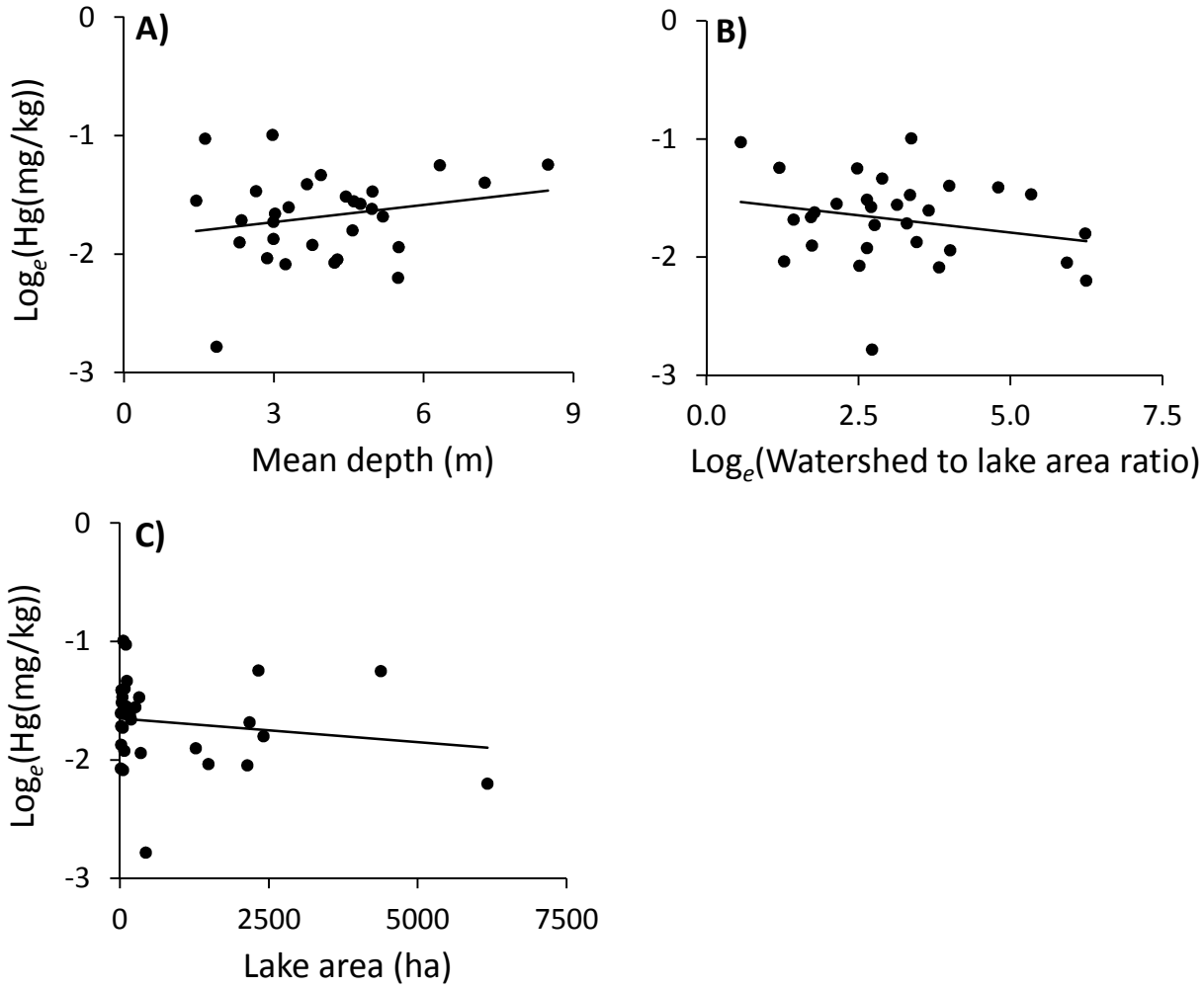

Figure 3.8. Mean log-transformed detected fish mercury concentrations (mg/kg) versus mean depth (A; m), log-transformed watershed to lake area ratio (B) and lake area (C; ha). Data points represent individual waterbodies.

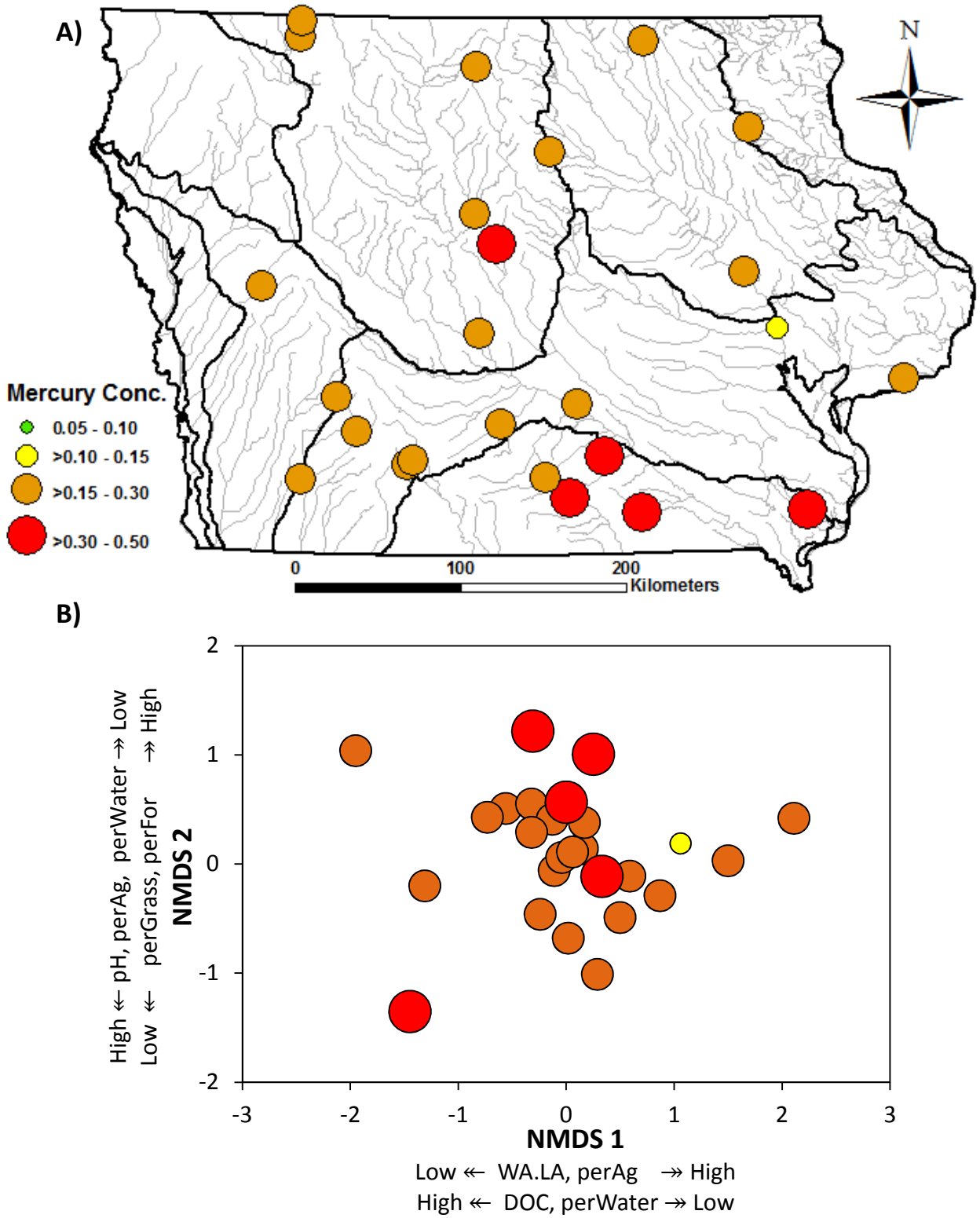

Figure 3.9. Mean detected largemouth bass mercury concentrations (mg/kg) by waterbody across Iowa (A) and plotted by an NMDS ordination based on the 10 variables identified to influence fish mercury concentrations (B). See Appendix B or Methods for variable abbreviations. Only variable vectors that were significantly correlated with NMDS axes scores are labeled on the axes ($r \geq 0.50$, $P < 0.05$).

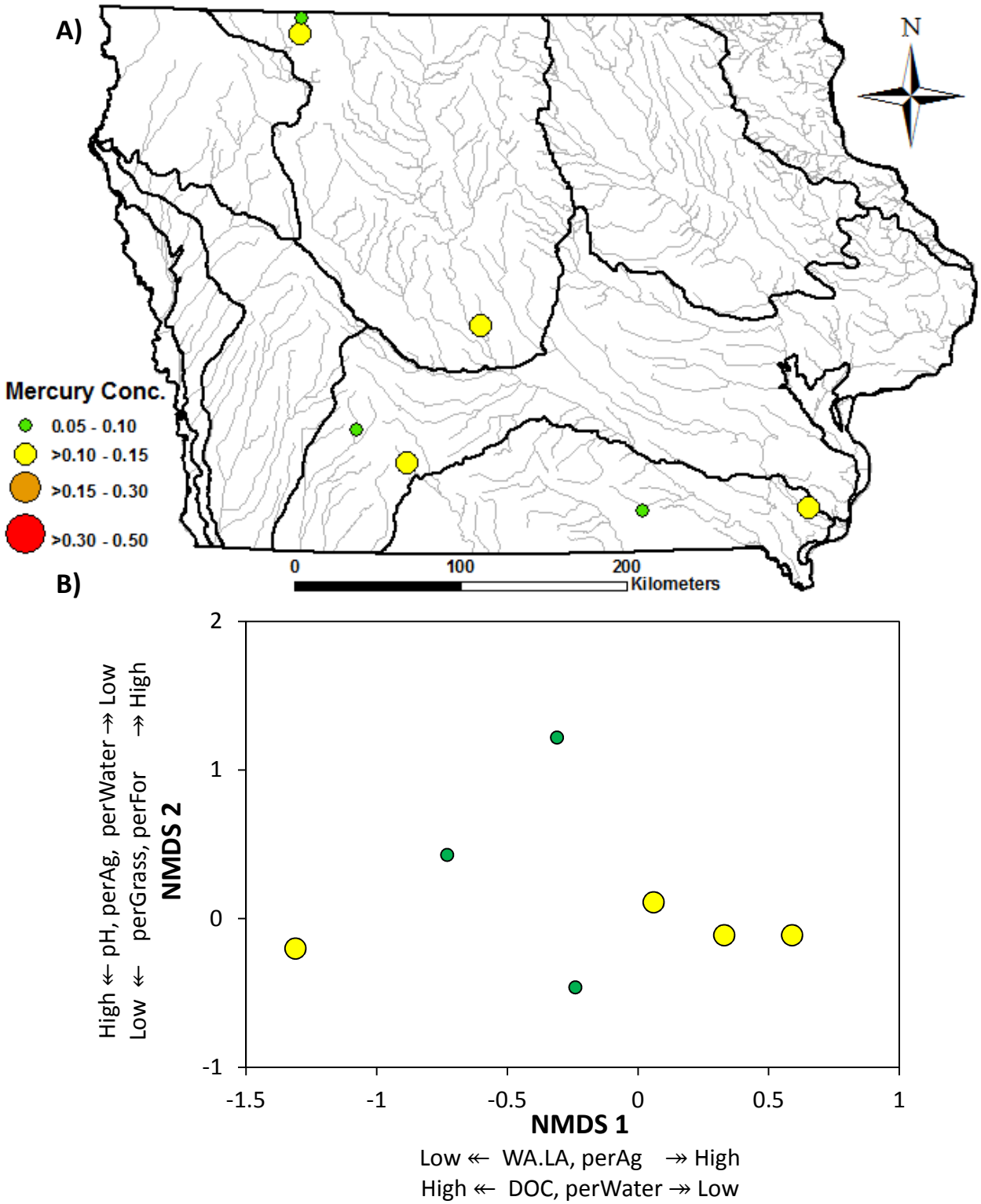

Figure 3.10. Mean detected bluegill mercury concentrations (mg/kg) by waterbody across Iowa (A) and plotted by an NMDS ordination based on the 10 variables identified to influence fish mercury concentrations (B). See Appendix B or Methods for variable abbreviations. Only variable vectors that were significantly correlated with NMDS axes scores are labeled on the axes ($r \geq 0.50$, $P < 0.05$).

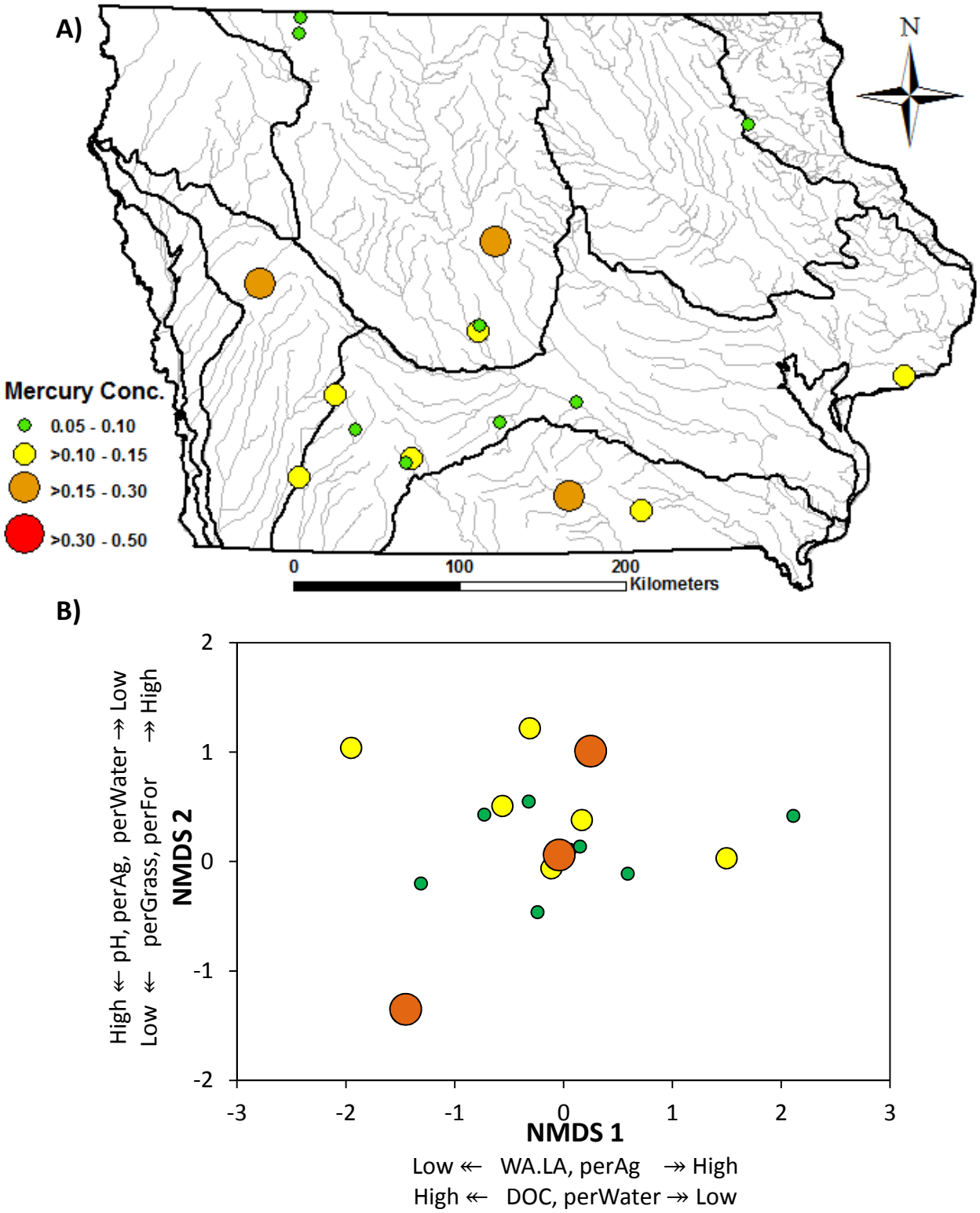

Figure 3.11. Mean detected black and white crappie (combined) mercury concentrations (mg/kg) by waterbody across Iowa (A) and plotted by an NMDS ordination based on the 10 variables identified to influence fish mercury concentrations (B). See Appendix B or Methods for variable abbreviations. Only variable vectors that were significantly correlated with NMDS axes scores are labeled on the axes ($r \geq 0.50$, $P < 0.05$).

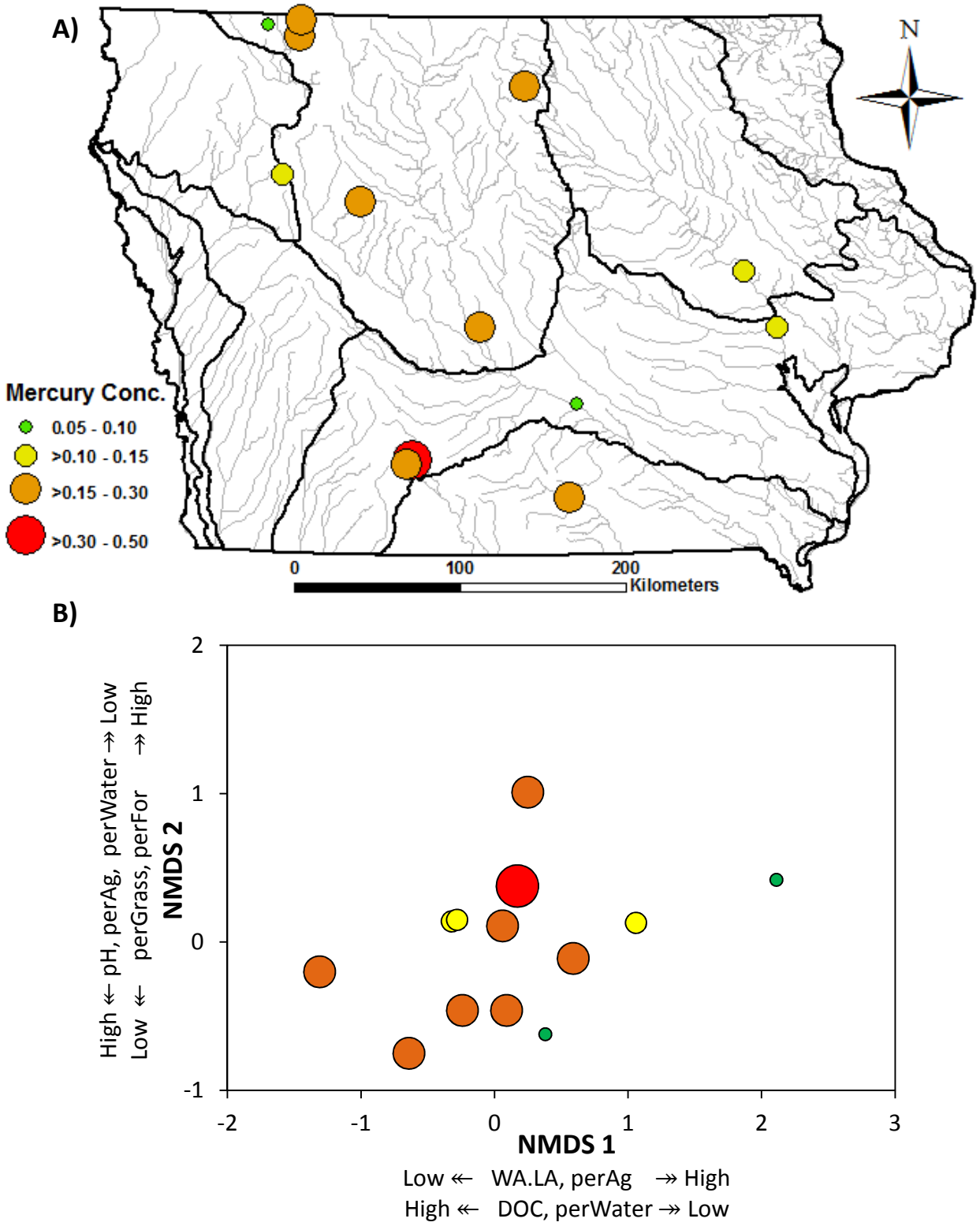

Figure 3.12. Mean detected walleye mercury concentrations (mg/kg) by waterbody across Iowa (A) and plotted by an NMDS ordination based on the 10 variables identified to influence fish mercury concentrations (B). See Appendix B or Methods for variable abbreviations. Only variable vectors that were significantly correlated with NMDS axes scores are labeled on the axes ($r \geq 0.50$, $P < 0.05$).

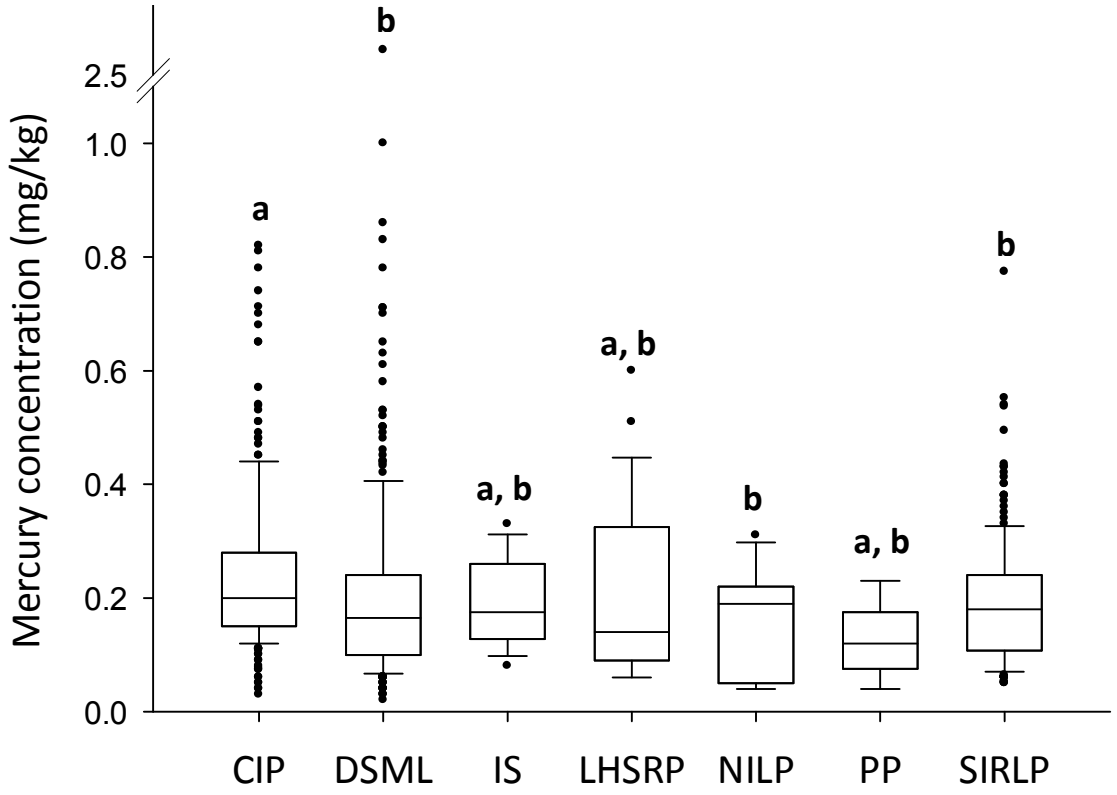

Figure 3.13. Box plots of detected fish mercury concentrations (mg/kg) by ecoregion (CIP = Central Irregular Plains; DSML = Des Moines Lobe; IS = Iowan Surface; LHSRP = Loess Hills and Steeply Rolling Prairies; NILP = Northwest Iowa Loess Prairies; PP = Paleozoic Plateau; SIRLP = Southern Iowa Rolling Loess Prairies). The line within the box represents the median value. Dimensions of the box represent the 25th and 75th percentiles. Error bars represent the 10th and 90th percentiles. Individual points are outliers. Boxes sharing a common letter are not significantly different (ANOVA; $P > 0.05$).

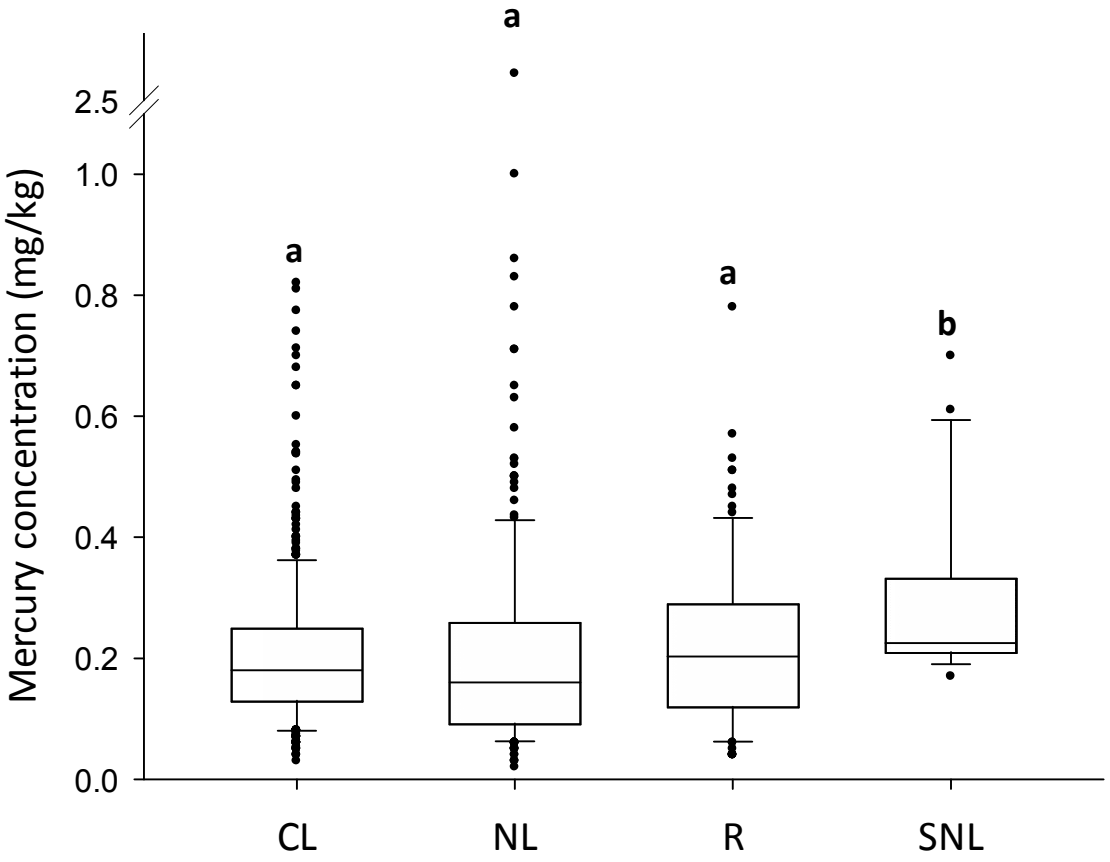

Figure 3.14. Box plots of detected fish mercury concentrations (mg/kg) by lake type (CL = constructed lake; NL = natural lake; R = reservoir; SNL = shallow natural lake). The line within the box represents the median value. Dimensions of the box represent the 25th and 75th percentiles. Error bars represent the 10th and 90th percentiles. Individual points are outliers. Boxes sharing a common letter are not significantly different (ANOVA; $P > 0.05$).

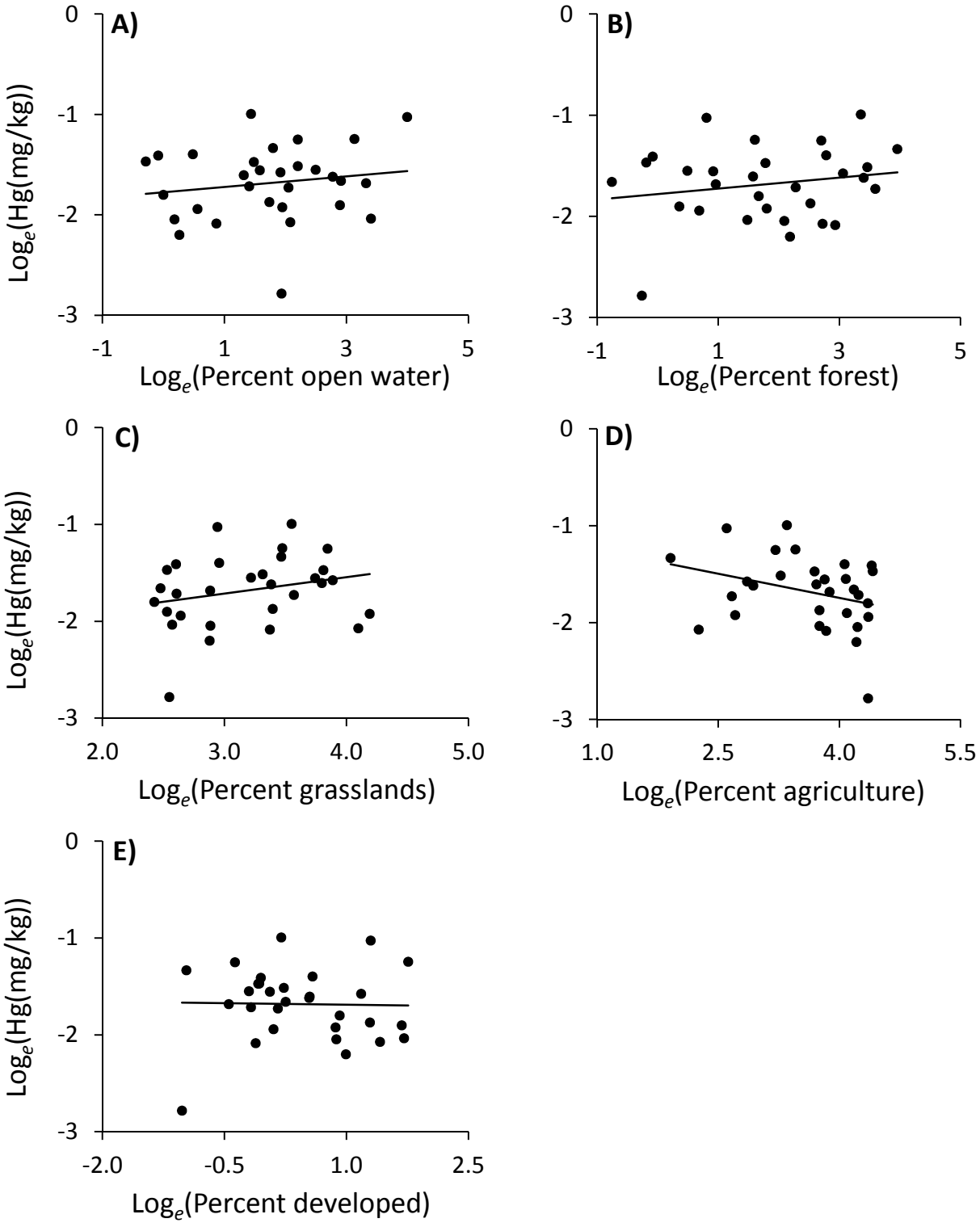

Figure 3.15. Mean log-transformed detected fish mercury concentrations (mg/kg) versus log-transformed watershed composition variables percent open water (A), percent forest (B), percent grasslands (C), percent agriculture (D), and percent developed (E). Data points represent individual waterbodies.

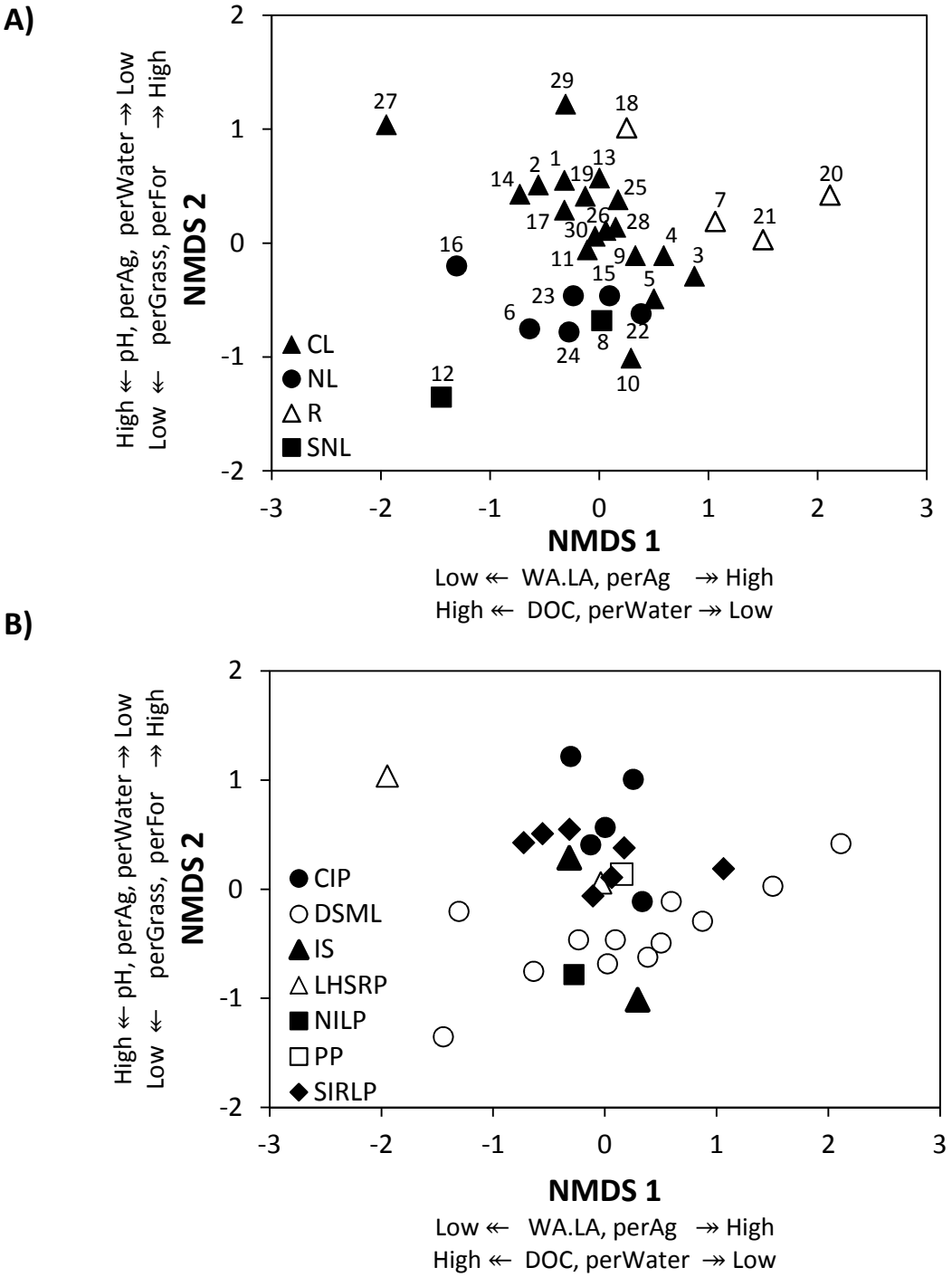

Figure 3.16. NMDS ordination of 30 Iowa lakes based on the 10 environmental variables identified to influence fish mercury concentrations. Lakes are coded by lake type (A; CL = constructed lake; NL = natural lake; R = reservoir; SNL = shallow natural lake) and ecoregion (B; CIP = Central Irregular Plains; DSML = Des Moines Lobe; IS = Iowan Surface; LHSRP = Loess Hills and Steeply Rolling Prairies; NILP = Northwest Iowa Loess Prairies; PP = Paleozoic Plateau; SIRLP = Southern Iowa Rolling Loess Prairies). Numbers (A) identify waterbodies listed in Table 3.1. See Methods or Appendix B for variable abbreviations.

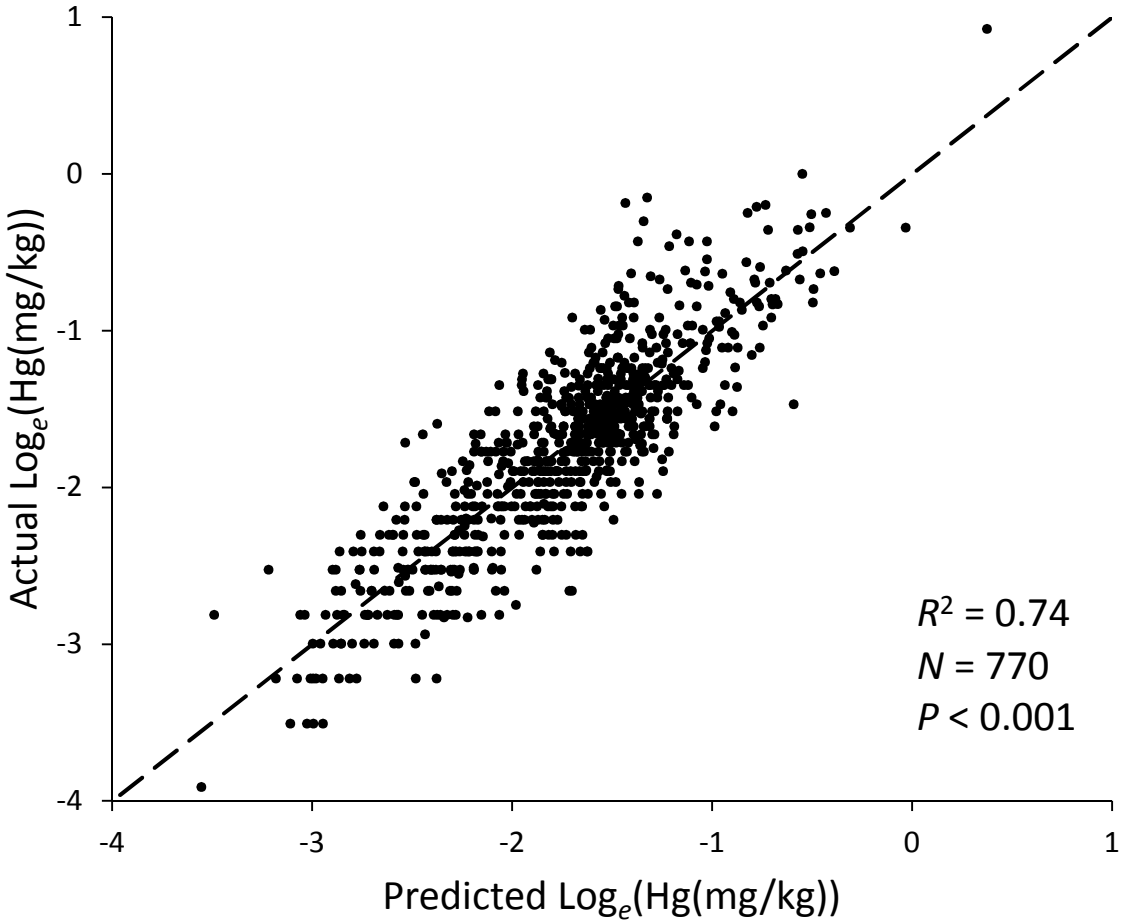

Figure 3.17. Predicted versus observed log-transformed fish mercury concentrations. The long dashed line represents the 1:1 line.

CHAPTER 4

FACTORS INFLUENCING FISH MERCURY CONCENTRATIONS IN IOWA INTERIOR RIVERS

Abstract

The presence of methylmercury in aquatic food webs has received much attention over the past couple of decades due to its health implications for those who consume contaminated fish. Mercury concentrations in fishes are highly variable within and among systems, influenced by a range of biotic and abiotic variables. However, predictive mercury models are region specific and factors influencing fish mercury concentrations in Iowa interior river systems are unknown. Channel catfish (*Ictalurus punctatus*, n = 205), flathead catfish (*Pylodictis olivaris*, n = 123), northern pike (*Esox lucius*, n = 60), smallmouth bass (*Micropterus dolomieu*, n = 176), and walleye (*Sander vitreus*, n = 176) were collected between March and October, 2014-2015, from ten Iowa interior rivers and tested for mercury contamination. Fish were collected from an upstream and a downstream location on six of the rivers to test for intra-river differences in fish mercury concentrations. Various land use, water chemistry, and fish characteristics were gathered and used to explain differences in mercury concentrations across and within Iowa interior rivers using multiple linear regression models sorted by AIC_c. Mercury concentrations were generally low (mean = 0.17 mg/kg, n = 740) but highest in flathead catfish, northern pike, smallmouth bass, and walleye and lowest in channel catfish. Fish mercury concentrations were positively related to fish length, age, trophic position, and $\delta^{13}C$ signatures. Abiotic variables

including phosphorus, sulfate, and percent of watershed as open water and grassland were negatively related to fish mercury concentrations, whereas hardness, nitrogen-ammonia, the Human Threat Index and percent of watershed as wetland and forested land were positively related to fish mercury concentrations. Additionally, fish collected from the Paleozoic Plateau ecoregion had higher mercury concentrations compared to those collected from other ecoregions. Together, these factors explained 70% of the variation in fish mercury concentrations. Results of this study suggest mercury concentrations are highest in large, old, piscivorous fishes inhabiting watersheds with low amounts of water, grasslands, and nutrients and higher amounts of wetlands and forests. This study provides a comprehensive analysis of abiotic and biotic factors influencing fish mercury concentrations in Iowa interior rivers and may have implications for consumption advisories.

Introduction

The presence of the neurotoxin methylmercury, hereon referred to as mercury, in aquatic food webs has received much attention over the past couple of decades because of its health implications for those who consume contaminated fish (Murata et al. 2006; Wentz et al. 2014). Numerous mercury monitoring programs have been developed to survey a variety of fish species and locations in order to develop fish consumption advisories (Wentz et al. 2014). While many factors have been suggested to influence fish mercury concentrations, an improved understanding of the determinants of mercury bioaccumulation and mercury cycling in the environment could help to guide mercury monitoring programs in predicting both sources and concentrations of mercury in fish.

Various abiotic and biotic factors have been related to mercury concentrations in freshwater fishes (Sackett et al. 2009; Rypel 2010; Tremain and Adams 2012). Fish mercury concentrations can vary among ecoregions (Sackett et al. 2009; Glover et al. 2010), whereas within ecosystem variation has been attributed to watershed composition and land use variables such as wetland area (Rypel 2010; Wentz et al 2014) and agricultural use (Benoit 2003). In addition to watershed-scale factors, biotic factors such as fish length, age, and trophic position can influence mercury concentrations (Rolfhus et al. 2011; Tremain and Adams 2012). With up to 94% of mercury in fish attributed to dietary sources (Phillips and Gregory 1979; Houck and Cech Jr. 2004; Pickhardt et al. 2006), there are distinct interspecific and intraspecific differences in mercury concentrations within fishes related to food web dynamics (Atwell et al. 1998; Sackett et al. 2009). As mercury bioaccumulates, larger and older piscivorous fishes tend to have high mercury concentrations compared to smaller planktivorous or insectivorous fishes (Olsson

1976; Mason et al. 1995; Wiener and Spry 1996; Tremain and Adams 2012). The use of nitrogen and carbon stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) provide a technique to precisely estimate trophic position and energy flow in fishes (Atwell et al. 1998). Nitrogen isotope ratios ($^{15}N/^{14}N$) allow determination of trophic position of organisms (Hesslein et al. 1991; Cabana and Rasmussen 1994; Post 2002) while carbon isotope ratios ($^{13}C/^{12}C$) help determine the source of dietary carbon and energy flow (Overman and Parrish 2001). Further, nitrogen isotope signatures ($\delta^{15}N$) have been positively related to fish mercury concentrations (Atwell et al. 1998) and may serve as a predictive tool for fish mercury concentrations.

Although a large body of literature exists evaluating the effects of biotic and abiotic factors on mercury concentrations of fish in lakes (Larsson et al. 1992; Pickhardt et al. 2002; Rypel 2010), few studies have comprehensively evaluated the influence of these factors on mercury concentrations in river fish (but see, Glover et al. 2010; Wentz et al. 2014). Despite the importance of lotic fisheries, relatively little is known about accumulation of mercury in Midwestern river fishes. Currently, certain states have conservatively implemented fish consumption advisories for reaches within interior river systems, instead of implementing advisories on entire rivers (IDNR 2014). Understanding whether or not differences in fish mercury accumulation exist within and among river systems will help to inform riverine fish consumption advisories.

Regional, local, and individual differences in abiotic (e.g., watershed composition and use, water chemistry, etc.) and biotic (e.g., length, age, species, trophic position) characteristics may explain much of the variability in mercury concentrations in lotic fishes. They may also play a large role in constructing predictive mercury accumulation models for application to regionally similar rivers that have not been evaluated. The objective of this study is to explore the influence

of a suite of abiotic and biotic factors on mercury concentrations in fishes of Iowa river systems. I hypothesized that variation in fish mercury concentrations would likely be explained by multiple abiotic and biotic factors including water chemistry, watershed composition, and individual fish characteristics.

Methods

Fish Collection & Processing

Channel catfish (*Ictalurus punctatus*, n = 205), flathead catfish (*Pylodictis olivaris*, n = 123), northern pike (*E. lucius*, n = 60), smallmouth bass (*Micropterus dolomieu*, n = 176), and walleye (*Sander vitreus*, n = 176) were collected between March and October 2014-2015, from four rivers in the Missouri River watershed (Missouri River, Little Sioux River, Rock River, and East Nishnabotna River) and eight rivers in the Mississippi River watershed (Mississippi River [pool 11 and 13], Skunk River, Iowa River, Cedar River, Des Moines River, Upper Iowa River, Maquoketa River, and Wapsipinicon River; Table 4.1) within Iowa. With the exception of the Skunk River, fishes sampled in the six main Mississippi tributaries were sampled from upstream and downstream locations in each river to evaluate spatial differences in mercury accumulation within river systems. Fish were collected upstream of Saylorville reservoir and downstream of Red Rock reservoir on the Des Moines River, upstream and downstream of Coralville reservoir on the Iowa River, upstream of Waterloo and downstream of Cedar Rapids on the Cedar River, upstream of Anamosa and downstream of Dixon on the Wapsipinicon River, upstream of Monticello and downstream of Maquoketa on the Maquoketa River, and upstream of Decorah and downstream near Dorchester on the Upper Iowa River (Figure 4.1). The goal was to sample

10-20 individuals of each species collected by 1 cm length groups (i.e., 1 individual/species/length group) at each sampling location. Substantial effort was made to collect a minimum of 10 fish of each species from each location, but fewer fish or duplicate length groups were used if 10 individuals could not be collected within a reasonable amount of sampling effort. Fishes were collected primarily with pulsed DC boat electrofishing but angling was used to supplement electrofishing catches when needed.

Soon after collection, fishes were measured for total length (TL mm) and weight (g) and then euthanized. Fish not processed immediately after capture were wrapped in aluminum foil, labeled with weight and length measurements, and frozen whole until processing. In the laboratory, sex was determined and aging structures applicable to each species were removed (e.g., sagittal otoliths for northern pike, smallmouth bass, and walleye, and lapilli otoliths for channel catfish and flathead catfish). All tissue samples were collected following USEPA fish tissue extraction protocols (USEPA 2000; USEPA 2003). Two 5-10 g samples of skinless dorsal axial muscle tissue were removed from each individual, one for mercury analysis and another for stable isotope analysis of $\delta^{15}N$ and $\delta^{13}C$. All tissue samples were removed wearing nitrile gloves and with a scalpel. Gloves were replaced and scalpels were thoroughly sanitized with 95% ethanol after each fish to avoid cross-contamination among samples. Tissue samples were stored in a -10°C freezer until transport for analysis.

Frozen fish tissue samples were transported on ice to the State Hygienic Lab (SHL), Ankeny, Iowa, for mercury analysis. Mercury contamination was determined using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) and reported as wet-weight total mercury concentrations (THg mg/kg; USEPA 1994). USEPA Method 200.8 for determination of mercury concentration procedures were followed to ensure quality assurance and control of all samples

(USEPA 1994). Mercury quantitation threshold is typically >0.05 mg/kg, but SHL reports detected fish mercury concentrations below 0.05 mg/kg when possible.

Otoliths were the primary aging structure for most sacrificed individuals (used for 99% of individuals), but pectoral (ictalurids) or dorsal (smallmouth bass and walleye) spines (1% of individuals) were used when otoliths were destroyed or unreadable. Otoliths and spines were cross-sectioned using a slow speed saw with a diamond wafering blade and pictures were taken under a microscope. Structures were aged at least two times by one reader without prior knowledge of fish size or capture location. Additional cross-sections were taken and ages were re-estimated when there were disagreements among age estimates.

Concurrently with fish sampling, mussels (e.g., plain pocketbook *Lampsilis cardium*) were collected from rivers for stable isotope analysis. All mussels were collected within 3 km of where fishes were sampled. These samples provide isotopic baseline data to standardize fish trophic position and energy flow estimates among systems (Overman and Parrish 2001). Approximately 40-50 personnel hours were spent sampling for freshwater mussels in rivers. I was able to collect 1-3 mussels from the upstream and downstream location on all six rivers where fish were collected, except for the downstream location on the Des Moines River. I also searched for mussels on the East Nishnabotna, Little Sioux, South Skunk, Missouri, and Mississippi (Pool 13) rivers. However, no mussels were found on the East Nishnabotna or Missouri rivers after searching for at least three hours at multiple locations on each river. Thus, baseline values of $\delta^{15}N$ or $\delta^{13}C$ could not be determined for the Rock and East Nishnabotna rivers. To include these rivers in the analysis, linear regression models between fish length and either trophic position or $\delta^{13}C$ were constructed to predict values for trophic position and $\delta^{13}C$ for each individual by species. Additionally, fishes collected from the Missouri River and UMR

(Appendix F) were removed from analysis because they did not have comparable water chemistry or land use data and are not considered interior river systems of Iowa.

A LaMotte zooplankton net was towed for 5-15 min. at most river locations. However, zooplankton abundances were extremely low in all but two locations and did not allow for an adequate sample (~1 g) to be collected for stable isotope analysis. Zooplankton samples were collected from the downstream locations on the Iowa and Des Moines rivers, both of which are likely to have derived from increased zooplankton productivity in major reservoirs (Coralville – Iowa; Red Rock – Des Moines) just upstream of the sampling reaches.

Fish and mussel tissue samples were dried in an oven at 50°C for 24-48 hours. Tissue samples were crushed to a fine powder with a mortar and pestle, and stored in glass scintillation vials. Approximately 1-2 μg of sample were folded in 7-mm tin capsules. Once in tin capsules, samples were transported to the Stable Isotope Laboratory on Iowa State University's campus for stable isotope analyses of $\delta^{15}N$ and $\delta^{13}C$. Samples were analyzed using a stable isotope mass spectrometer and isotopic signatures are reported in parts per thousand using the following equation (Atwell et al. 1998):

$$\delta X = [R_{sample}/R_{standard}) - 1] \times 1000$$

where X is $\delta^{15}N$ or $\delta^{13}C$ and R is the ratio $^{15}N:^{14}N$ or $^{13}C:^{12}C$ (Atwell et al. 1998). Fish trophic position was calculated using the following formula developed by Cabana and Rasmussen (1996):

$$\text{Trophic position} = [(\text{fish } \delta^{15}N - \text{mussel } \delta^{15}N)/3.4] + 2$$

Watershed and Water Chemistry Data

Water quality and water chemistry data were extracted from the Iowa Department of

Natural Resources (IDNR) Ambient Stream Monitoring program online database (IDNR 2015). Data extracted from this database included analytes that were present for all stream monitoring sites. Analytes included in the database are hardness ($CaCO_3$ mg/L), nitrate + nitrite (NN; mg/L), nitrogen (ammonia; N.A; mg/L), orthophosphate (ortho; mg/L), pH, phosphate-phosphorous (phos; mg/L), dissolved solids (DS; mg/L), total suspended solids (TSS; mg/L), total volatile suspended solids (TVSS; mg/L), and sulfate (mg/L). Water samples were collected by the IDNR monthly from each of 91 river monitoring sites throughout the state. Water quality variables were averaged for individual fish based upon the age of each fish. For example, a 3-year-old fish collected in 2015 had water quality metrics averaged for years 2012-2015. Upstream and downstream river monitoring sites were only available on the Iowa River, Des Moines River, Cedar River, and Wapsipinicon River. Water quality metrics were used from one sampling for fishes collected form upstream and downstream locations on the Upper Iowa and Maquoketa rivers. River watershed area (km^2) was determined using ArcGIS software. Watershed data were extracted from the Human Threat Index (HTI) database developed by Annis et al. (2010). Information compiled for each river sampling site included watershed land use variables such as open water (%), wetland area (%), grassland area (%), forested land (%), row-crop agricultural (%), developed land (%), and barren land (%). Other variables extracted from the HTI database included stream order and three HTI values; a local HTI value, a watershed HTI value, and an overall HTI value. HTI values are on a scale of 0-100 and are assigned based on how impacted the stream segment is with higher values indicating a higher human impact on the stream segment (Annis et al. 2010). Impact assessment is based on impervious surfaces, landfills, dams, mining operations, agriculture, and other forms of anthropogenic disturbance within a watershed.

Statistical Analyses

The initial set of variables was reduced by eliminating correlated variables that represent similar attributes (Appendix G and H). Variables eliminated during this process included orthophosphate, total volatile suspended solids, and local and watershed HTI. Phosphate-phosphorous and orthophosphate were highly correlated and represented similar measures of nutrients. Thus, orthophosphate was eliminated. Total suspended solids and total volatile suspended solids were highly correlated and represent similar measures of particulates in the water column. Thus, the total volatile suspended solids variable was eliminated. Both local HTI and watershed HTI were highly correlated with overall HTI values. Overall HTI represents a combination of local and watershed HTI (Annis et al. 2010), and thus the local and watershed HTI variables were removed from further analysis to reduce redundancy in the explanatory variables. Additionally, percent barren land was eliminated because it was not hypothesized to affect fish mercury concentrations.

Similarities and differences among rivers based on environmental characteristics were explored using nonmetric multidimensional scaling (NMDS) ordination. The remaining 17 environmental variables (water chemistry [15 year average], watershed characteristics, and composition data) were first normalized (mean = 0, standard deviation = 1), a 16 x 16 Euclidean Distance matrix was calculated, and the matrix was used as input to the NMDS ordination. Rivers were plotted by ecoregion to show similarities and differences among rivers within ecoregions with vectors labelled along the axes indicating variable influences on spatial orientation within the ordination space. The normalization, distance matrix, NMDS ordination and vectors were generated using PRIMER (Clarke and Gorley 2006).

Of the 740 observations in the dataset, only three had undetected mercury concentrations (reported as <0.05 mg/kg from SHL) and were assigned a value of half the detection limit (0.025 mg/kg). Additionally, 13 observations had reported values less than the detection limit. For example, three observations were reported as 0.03 mg/kg, and 10 observations were reported as 0.04 mg/kg from SHL. These values were not altered for the following analyses.

Building the predictive model

In addition to the 26 individual abiotic and biotic variables of interest (Appendix G), interaction terms between variables were added and tested when appropriate and log-transformed certain variables to normalize the residuals. For example, interaction terms between species-age, species-sex, species-length, and sex-length were added to allow for different slopes between these variables and fish mercury concentrations. Fish mercury concentrations and percent watershed composition variables were log-transformed to normalize residuals.

A model selection procedure was used to evaluate a suite of variables for predicting fish mercury concentrations. Regression subset selection, hereon referred to as "regsubsets", under the R-package, "leaps", was used to evaluate all model combinations in two parts (Thomas Lumley using Fortran code by Alan Miller 2009; R Core Team 2016): fish-level variables (biotic and water chemistry data) and river-level variables (river specific information and watershed composition data). First, regsubsets on was conducted on fish-level variables with a fixed effect on the 'River' term. The 'River' variable is a term unique to each river sampling location, and is used to account for variation in fish mercury concentrations among rivers while regsubsets selects models that explain the most variation in fish mercury concentrations using fish-level variables. Models were created using the exhaustive model selection procedure (all model

combinations) and sorted using Akaike's Information Criterion (AIC_c) as the model selection criterion (Burnham and Anderson 1998). Variables and interactions included in the top model were retained when evaluating river-level variables. All other fish-level variables were removed from the model for the succeeding steps in the analysis.

Next, another exhaustive regsubsets model selection procedure was used on river-level variables to determine if any additional variation in fish mercury concentrations could be explained by river specific characteristics. For this step, the 'River' term was omitted and the retained fish-level variables, determined in the previous step, were forced into the model selection procedure. Using the results of the top AIC_c models from each model selection procedure, a final multiple linear regression model was created to predict fish mercury concentrations, and to describe variation in fish mercury concentrations within and across Iowa interior river systems.

An NMDS ordination was then created using only the environmental variables that were identified to predict fish mercury concentrations in the final predictive model. Additional plots show relative differences in mean mercury concentration between lakes as different sized points in ordination space. Finally, analysis of variance (ANOVA) was conducted for all categorical variables identified to influence fish mercury concentrations.

Results

Ordination #1

Two-dimensional ordination of sampling locations based on 17 environmental variables had a stress value of 0.14, indicating the ordination provides a useful picture of similarities and

differences among locations, but that the precise positioning of sampling locations in ordination space should be interpreted with caution (Clarke and Warwick 2001). When coded by ecoregion, the ordination indicated similarities within ecoregions (Figure 4.2). The two sampling locations in the DSML ecoregion grouped to the far upper right based on their large watersheds, higher percentages of agriculture, wetlands, developed land, and open water in their watersheds, high sulfates, high dissolved solids, and high HTI values. The downstream sampling location on the Upper Iowa River (13; PP ecoregion) is positioned in the far left of the figure based on high percentages of grasslands and forested land and low percentage of agriculture. Sampling locations in the IS ecoregion are spread throughout the center of the figure indicating intermediate values of the variables contributing to the ordination. The Little Sioux and Rock rivers (NILP ecoregion) are positioned in the lower right corner of the figure based on high percentage of agriculture in the watershed and high dissolved and total suspended solids and hardness. The four sampling locations within the SIRLP ecoregion grouped in the middle of the ordination along the NMDS 2 axis, but were spread out along the NMDS 1 axis indicating uniformly intermediate values of the variables influencing NMDS 2 and variability in factors influencing NMDS 1. Finally, the East Nishnabotna River (LHSRP ecoregion) is positioned in the bottom of figure due primarily to high total suspended solids (Figure 4.2)

Fish-level factors

Channel catfish were the most ubiquitous species among the rivers and were collected from 8 out of 10 rivers sampled (Table 4.2). Smallmouth bass and walleye were collected from both upstream and downstream locations on the six main Mississippi tributaries (Table 4.3). Flathead catfish were sampled from two of the six upstream sites and five of the six downstream

sites (Table 4.3). Only smallmouth bass and walleye were collected from the Upper Iowa River. Northern pike relative abundance in interior rivers was generally low, making fish collection difficult. Northern pike were sampled from the downstream location on the Des Moines River and the upstream locations on the Iowa, Cedar, and Wapsipinicon rivers (Table 4.3).

The top fish-level AIC_c model contained the main effects of fish species, sex, age, length, $\delta^{13}C$, trophic position, water hardness, nitrogen-ammonia, phosphorous, total suspended solids, and sulfate ($w_i = 0.38$; Table 4.4). Among the top ten fish-level AIC_c models evaluated, all contained biotic variables fish species, age, $\delta^{13}C$, and length, suggesting these variables are important predictors of fish mercury concentrations. Variation in the presence of water chemistry variables accounted for the majority of the differences among models (Table 4.4). Though not found in the top AIC_c model, Julian day, dissolved solids, and pH were retained in many of the models that received some support (AIC_c <2, w_i = > 0.10).

Across all rivers, the highest mercury concentrations were found in flathead catfish, northern pike, smallmouth bass, and walleye with lower concentrations observed for channel catfish (Table 4.5; Figure 4.3; ANOVA; $P < 0.05$). Mean mercury concentrations of all fishes was less that the EPA criterion of 0.30 mg/kg and only 10% of the fishes collected from interior rivers had mercury concentrations at or above 0.30 mg/kg. The highest measured mercury concentration reported was 0.86 mg/kg, measured in a 477 mm, 9-year-old smallmouth bass collected from the Upper Iowa River. Only 5 of 205 channel catfish samples (<3%) exceeded the advisory limit. Northern pike mercury concentrations were generally low, with only 1 out of 60 samples exceeding the advisory limit.

Mercury concentrations across all species were similar between males and females but lower when sex was unknown (Table 4.6; Figure 4.4 and 4.5; ANOVA; $P = 0.05$). Mercury

concentrations increased with fish age and length but, based on R^2 values, age explained more variation in fish mercury concentrations than length for flathead catfish, northern pike, smallmouth bass, and walleye (Table 4.5; Appendix L). Trophic position was also positively related to mercury concentrations in flathead catfish, smallmouth bass, and walleye but not in channel catfish and northern pike (Table 4.5). Across all species, trophic position was positively related to fish mercury concentrations (Table 4.7; Figure 4.6) and explained more variation than $\delta^{13}C$ signatures in all species except channel catfish and walleye (Table 4.5). However, $\delta^{13}C$ was positively related to fish mercury concentrations when all species were combined (Table 4.5 and 4.7; Figure 4.6).

Relationships between fish mercury concentrations and water chemistry variables were highly variable (Figure 4.7). Mercury concentrations were positively related to nitrogen-ammonia (95% CI of slope: 2.50 to 5.06) and water hardness (95% CI of slope: 0.004 to 0.01) and negatively related to phosphorous (95% CI of slope: -1.40 to -0.45) and sulfates (95% CI of slope: -0.03 to -0.01; Table 4.7; Figures 4.7). The slope of total suspended solids did not differ from zero (-0.002 to 0.001; Table 4.7; Figures 4.7).

River-level factors

The top river-level AIC_c model contained HTI, ecoregion, and percent watershed composition variables open water, wetlands, forested land, and grassland area ($w_i = 0.50$; Table 4.8). Though not found in the top model, watershed area and stream order were in many of the models that received support ($AIC_c < 2$; $w_i > 0.10$; Table 4.8). None of the top ten AIC_c models contained the categorical upstream/downstream variable, suggesting no difference in fish

mercury concentrations between upstream and downstream locations across Iowa interior rivers (Table 4.8).

HTI values were positively related to mercury concentrations in the final predictive model (Table 4.7) but biplots indicated a negative relationship (Figure 4.8). This is likely due to other variables influencing the parameter estimate in the model. Fish mercury concentrations were higher in the PP ecoregion compared to other ecoregions (Table 4.9; Figure 4.9; ANOVA; $P < 0.01$). Percent of watershed as open water and grasslands were negatively related to fish mercury concentrations while percent of watershed as forested land and wetland area was positively related to fish mercury concentrations (Table 4.7; Figure 4.10). The final multiple regression model based on variables included in the top fish-level and river-level AIC_c models explained 70% of the variation in fish mercury concentrations across Iowa interior rivers ($R^2 = 0.70$; Table 4.7; Figure 4.11). The model tended to overestimate low mercury concentrations and under estimate higher mercury concentrations (Figure 4.11).

Ordination #2

Two-dimensional ordination of rivers based on the 10 environmental variables identified to predict fish mercury concentrations had a similar stress value of 0.12 (Figure 4.2). Variables influencing NMDS 1 and 2 were similar to that of the 17 variable ordination (Figure 4.2). Fish sampling locations positioned in the top portion of the ordination have high phosphorous and total suspended solids. Fish sampling locations positioned to the left have watersheds with high percentages of forested land and grassland area whereas fish sampling locations towards the right have watersheds with high percentages of open water and wetlands, higher water hardness, and high sulfates. Mean mercury concentrations by river sampling location for all species were

variable throughout the ordination space and no apparent spatial patterns were observed (Figures 4.12-16).

Discussion

Results of this study suggest a suite of biotic and abiotic factors are associated with differences in fish mercury concentrations in Iowa rivers. With varying degrees of effects, fish length, age, species, trophic position, carbon signature, sex, water chemistry, ecoregion, watershed area, and land use explained 70% of the variation in fish mercury concentrations in fishes collected from Iowa rivers. Fish mercury concentrations were positively related to fish length and age, suggesting that larger and older fishes have higher mercury concentrations, which is consistent with results from previous evaluations (e.g., Phillips et al. 1980; Eagles-Smith et al. 2008; Sackett et al. 2009). Thus, fish size may be one tool to help guide mercury consumption advisories.

Mercury concentrations varied among species. Of the five species of interest, channel catfish had the lowest fish mercury concentrations and mean trophic position, likely due to omnivorous feeding habits (Tyus and Nikirk 1990). Additionally, northern pike have surprisingly low mercury concentrations in Iowa interior rivers compared to Iowa lakes whereas walleye mercury concentrations were similar between systems (Chapter 3). Lower mercury concentrations of northern pike in rivers may be because they tend to prey upon fishes at lower trophic levels. Northern pike in Iowa lakes feed primarily on centrarchids (sunfishes), juvenile walleye, and freshwater drum (*Aplodinotus grunniens*; E. Ball, Iowa State University, unpublished data), whereas northern pike in Iowa rivers would likely be feeding on catostomid

and cyprinid (suckers and minnows) species (N. Mills, unpublished data), as they are the most abundant potential prey source in non-wadable Iowa rivers (Parks et al. 2014). However, neither $\delta^{13}C$ signatures nor trophic position were significantly related to lotic northern pike mercury concentrations.

Although $\delta^{13}C$ signatures were not correlated with mercury concentrations for most species in this analysis, $\delta^{13}C$ signatures were positively related to fish mercury concentrations and a positive relationship between walleye mercury concentrations and $\delta^{13}C$ signatures was observed, suggesting mercury concentrations are higher in fishes that rely more on allochthonous energy sources. In contrast, lake trout (*Salvelinus namaycush*) mercury concentrations were inversely related to $\delta^{13}C$ signatures, suggesting higher mercury concentrations in fishes with pelagic food source dependent diets (Power et al. 2002).

Fish trophic position was also positively related to overall fish mercury concentrations. However, mercury concentrations were generally unrelated to trophic position for individual species. Prey items consumed by river fishes may have highly variable mercury concentrations, which may explain why trophic position and $\delta^{13}C$ do not explain much of the variation in fish mercury concentrations. Additional sampling of common catostomid or cyprinid species from Iowa rivers for mercury and stable isotope analysis may provide additional insight into food web dynamics and mercury bioaccumulation across species with different trophic positions.

Beyond fish-specific characteristics, mercury concentrations varied among ecoregions. Fish collected from the Upper Iowa River (Paleozoic Plateau ecoregion) had higher mercury concentrations compared to other rivers throughout the state. Specifically, average mercury concentrations were about twice as high in walleye and smallmouth bass collected from the Upper Iowa River compared to all other river sampling locations. The soils and topography of

the Paleozoic Plateau ecoregion are unique and are considered “Loess with Bedrock Outcrops” that are defined by steeply sloped rolling hills and bluffs created from leoss and abundant emergent bedrock in the form of limestone (USDA 1997). The loess rich soils of this region are similar to the loess soils found in other regions of the state. However, the heavily forested and steep terrain of the watersheds make this region of the state unique and is likely contributing to increased mercury levels in the region.

The least agriculturally impacted watersheds of Iowa are found in the northeastern portion of the state where the Upper Iowa River is located. Though agricultural land and forested land are strongly negatively correlated (r = -0.96, $P < 0.01$; Appendix H), increases in agricultural land within a watershed likely does not directly influence fish mercury concentrations. Riparian zones adjacent to streams can provide favorable conditions for methylation productivity (Skyllberg et al. 2003). Further, the organic soil layers of forested land can harbor mercury fixing bacteria (Matilainen et al. 2000). This suggests that watersheds with higher percentages of forested land, particularly riparian forests, are contributing more bio-available mercury into aquatic systems compared to watersheds with relatively low percentages of forested land. Thus, the removal or retention of forested land that harbor mercury fixing bacteria likely contributes to decreases or increases in fish mercury concentrations. Further, these results suggest that less impacted river watersheds tend to have fish with higher mercury concentrations.

Reduced nutrient and sediment runoff in northeastern Iowa is likely due to smaller percentages of agriculture in these watersheds. Agricultural practices, such as fertilizer application and removal of riparian buffer zones, are associated with increases in nutrient and sediment runoff (Olness et al. 1975; Lowrance et al. 1984). The percentage of agriculture in the

watershed appeared in the second and third most supported model and agricultural land use is negatively related to mercury concentrations in lentic fishes in Iowa (Chapter 3). With approximately 86% of the total land use in Iowa devoted to agriculture, many watersheds are agriculturally dominated (USDA 2014) that may result in low mercury concentrations generally observed throughout the state compared to regions of North America (Kamman et al. 2005).

Several water chemistry metrics related to agricultural land use, including hardness, nitrate-ammonium, phosphorus, total suspended solids, and sulfate were also related to mercury concentrations. However, I advise interpreting water chemistry impacts on fish mercury concentrations with caution. All downstream locations on these sites were openly connected to the Mississippi River. It is assumed that fish collected in sampling reaches were resident fish, meaning they lived most of their life in the sampling reach and experienced only the water quality that was used in this analysis. However, there is considerable fish movement within and between these interior rivers and the Mississippi and Missouri rivers (Ickes et al. 2001). I acknowledge this aspect of the analysis as a caveat of the data used, but I believe the results of these analyses still provide a useful representation of how water quality can impact fish mercury concentrations in Iowa rivers.

This study provides a comprehensive analysis of factors influencing fish mercury concentrations in Iowa interior rivers and also serves as further evidence to suggest fish mercury concentrations are influenced by a suite of abiotic and biotic factors within and across systems. However, an important component of any predictive model is model validation. Additional sampling of fishes from rivers throughout the state, particularly rivers differing in watershed characteristics, would help validate this model and its usefulness as a predictive tool.

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Tables and Figures

Table 4.1. Characteristics of 16 reaches in 10 Iowa rivers examined in this study. U = upstream sampling location, D = downstream sampling location. ID refers to the identification number found in Figure 4.1. Ecoregions: DSML = Des Moines Lobe; IS = Iowan Surface; NILP = Northwest Iowa Loess Prairies; PP = Paleozoic Plateau; SIRLP = Southern Iowa Rolling Loess Prairies. SO = stream order; WA = watershed area (km^2); HTI = Human Threat Index. Land use variables are percentages of the watershed area; perWater = percent open water; perWet = percent wetland area; perFor = percent forested land; perGrass = percent grassland area; perAg = percent row crop agriculture; perDev = percent developed land.

River (U/D)	ID	Ecoregion	SO	WA	HTI	perWater	perWet	perFor	perGrass	perAg	perDev
Cedar (D)	1	IS	6	17568	52	1.0	0.5	7.2	14.9	72.9	3.1
Cedar (U)	2	IS	6	6268	68	1.2	0.6	6.2	14.8	73.9	2.9
Des Moines (D)	3	DSML	7	33223	67	1.4	1.2	12.4	20.6	61.2	2.6
Des Moines (U)	4	DSML	6	12226	50	1.0	1.7	5.3	11.3	77.8	2.5
East Nishnabotna	5	LHSRP	5	2971	54	0.8	0.2	4.8	22.2	69.5	2.0
Iowa (D)	6	SIRLP	6	12396	51	1.2	1.1	9.0	20.5	65.1	2.5
Iowa (U)	7	SIRLP	6	8044	49	1.2	1.4	8.1	17.9	68.4	2.4
Little Sioux	8	NILP	6	6492	50	1.9	0.7	5.1	17.2	72.6	2.0
Maquoketa (D)	9	SIRLP	6	4834	47	0.6	0.2	12.9	23.4	59.8	2.3
Maquoketa (U)	10	IS	5	2423	50	0.6	0.2	12.0	19.7	64.6	2.3
Rock	11	NILP	6	1998	43	0.5	0.6	1.4	11.4	82.8	2.3
Skunk	12	SIRLP	6	11228	52	0.8	0.8	11.4	21.0	62.5	2.5
Upper Iowa (D)	13	PP	5	2025	39	0.6	0.4	22.1	29.7	44.0	2.3
Upper Iowa (U)	14	IS	4	764	52	0.5	0.5	11.5	25.1	59.5	2.3
Wapsipinicon (D)	15	IS	5	6557	48	0.8	0.5	9.1	14.7	72.2	2.3
Wapsipinicon (U)	16	IS	5	4018	53	0.7	0.4	8.8	14.9	72.5	2.4

Table 4.2. Mean fish mercury concentrations (Hg; mg/kg), standard deviation (SD), and sample size (n) by species on ten rivers throughout Iowa. CHC = channel catfish, FHC = flathead catfish, NOP = northern pike, SMB = smallmouth bass, WAE = walleye.

River	Metric	CHC	FHC	NOP	SMB	WAE	All species
Cedar	Mean Hg	0.10	0.14	0.19	0.13	0.18	0.14
	SD	0.05	0.10	0.06	0.08	0.08	0.08
	n	42	20	9	32	32	135
Des Moines	Mean Hg	0.13	0.17	0.14	0.14	0.14	0.15
	SD	0.10	0.15	0.05	0.03	0.15	0.12
	n	38	35	18	6	33	130
East Nishnabotna	Mean Hg	0.10	0.27	-	-	-	0.15
	SD	0.07	0.13	-	-	-	0.12
	n	19	8	0	0	0	27
Iowa	Mean Hg	0.14	0.17	0.13	0.19	0.15	0.16
	SD	0.10	0.13	0.05	0.14	0.08	0.11
	n	24	32	14	34	32	136
Little Sioux	Mean Hg	0.15	-	-	-	0.19	0.18
	SD	0.09	-	-	-	0.07	0.08
	n	7	0	0	0	12	19
Maquoketa	Mean Hg	0.10	0.12	0.17	0.15	0.17	0.14
	SD	0.05	0.06	0.08	0.05	0.09	0.07
	n	29	11	4	27	18	89
Rock	Mean Hg	-	-	-	0.19	-	0.19
	SD	-	-	-	0.03	-	0.03
	n	0	0	0	10	0	10
Skunk	Mean Hg	0.14	0.24	-	-	-	0.16
	SD	0.12	0.06	-	-	-	0.11
	n	12	3	0	0	0	15
Upper Iowa	Mean Hg	-	-	-	0.29	0.30	0.29
	SD	-	-	-	0.20	0.14	0.17
	n	0	0	0	32	27	59
Wapsipinicon	Mean Hg	0.11	0.23	0.15	0.21	0.26	0.19
	SD	0.06	0.22	0.07	0.09	0.11	0.12
	n	34	14	15	35	22	120

Table 4.3. Mean fish mercury concentrations (Hg; mg/kg), standard deviation (SD), and sample size (n) by species and upstream (U) and downstream (D) locations on six rivers. CHC = channel catfish, FHC = flathead catfish, NOP = northern pike, SMB = smallmouth bass, WAE = walleye.

River (U/D)	Metric	CHC	FHC	NOP	SMB	WAE	All Species
Cedar (D)	Mean Hg	0.08	0.14	-	0.14	0.22	0.13
	SD	0.04	0.10	-	0.07	0.05	0.08
	n	22	20	0	15	12	69
Cedar (U)	Mean Hg	0.11	-	0.19	0.13	0.16	0.14
	SD	0.05	-	0.06	0.10	0.09	0.08
	n	20	0	9	17	20	66
Des Moines (D)	Mean Hg	0.13	0.21	0.14	0.13	0.13	0.15
	SD	0.13	0.14	0.05	-	0.15	0.13
	n	20	17	16	1	18	72
Des Moines (U)	Mean Hg	0.12	0.14	0.14	0.14	0.16	0.14
	SD	0.07	0.16	0.00	0.04	0.15	0.12
	n	18	18	2	5	15	58
Iowa (D)	Mean Hg	0.10	0.18	0.19	0.12	0.09	0.13
	SD	0.05	0.15	-	0.03	0.02	0.10
	n	11	22	1	13	16	63
Iowa (U)	Mean Hg	0.17	0.16	0.13	0.23	0.22	0.19
	SD	0.12	0.09	0.05	0.17	0.07	0.12
	n	13	10	13	21	16	73
Maquoketa (D)	Mean Hg	0.13	0.12	0.17	0.15	0.14	0.14
	SD	0.07	0.06	0.08	0.05	0.03	0.06
	n	13	11	4	14	7	49
Maquoketa (U)	Mean Hg	0.08	-	-	0.15	0.19	0.13
	SD	0.02	-	-	0.06	0.11	0.08
	n	16	0	0	13	11	40
Upper Iowa (D)	Mean Hg	-	-	-	0.35	0.28	0.32
	SD	-	-	-	0.21	0.12	0.17
	n	0	0	0	19	18	37
Upper Iowa (U)	Mean Hg	-	-	-	0.21	0.34	0.26
	SD	-	-	-	0.15	0.17	0.17
	n	0	0	0	13	9	22
Wapsipinicon (D)	Mean Hg	0.10	0.23	0.12	0.18	0.21	0.17
	SD	0.05	0.22	0.03	0.10	0.04	0.13
	n	16	14	3	15	10	58
Wapsipinicon (D)	Mean Hg	0.13	-	0.15	0.24	0.30	0.20
	SD	0.07	-	0.08	0.08	0.14	0.11
	n	18	0	12	20	12	62

Table 4.4. Top ten multiple regression models developed to predict fish mercury concentrations using fish-level variables (see Methods) ordered by Akaike's information criterion (AIC_c) using regression subset selection procedure. K = the number of parameters in the model (includes Waterbody), ΔAIC_c = the distance of each model from the best AIC_c model, and w_i = the model weight (a measure of relative strength). Each model was produced from 740 observations.

Model	K	AIC_c	ΔAIC_c	w_i
Species, Sex, Age, TL, $\delta^{13}C$, TP, Hardness, N.A, Phos, TSS, Sulfate, Species*Age, Species*Sex, Age*TL	28	-794.93	0.00	0.38
Species, Sex, Age, TL, $\delta^{13}C$, TP, Hardness, N.A, Phos, Sulfate, Species*Age, Species*Sex, Age*TL	27	-794.17	0.76	0.26
Species, JD, Sex, Age, TL, $\delta^{13}C$, TP, Hardness, N.A, Phos, TSS, Sulfate, Species*Age, Species*Sex, Age*TL	30	-793.67	1.26	0.20
Species, Sex, Age, TL, $\delta^{13}C$, TP, Hardness, N.A, DS, TSS, Species*Age, Species*Sex, Age*TL	26	-792.50	2.42	0.11
Species, JD, Sex, Age, TL, $\delta^{13}C$, TP, Hardness, N.A, pH, Phos, DS, TSS, Sulfate, Species*Age, Species*Sex, Age*TL	33	-789.70	5.23	0.03
Species, Sex, Age, TL, $\delta^{13}C$, Hardness, N.A, DS, TSS, Species*Age, Species*Sex, Age*TL	25	-788.22	6.71	0.01
Species, Age, TL, $\delta^{13}C$, TP, Hardness, N.A, DS, TSS, Species*Age, Age*TL	23	-779.67	15.26	0.00
Species, JD, Sex, Age, TL, $\delta^{13}C$, TP, Hardness, NN, N.A, pH, Phos, DS, TSS, Sulfate, Species*Age, Species*Sex, Age*TL	40	-776.82	18.11	0.00
Species, Age, TL, $\delta^{13}C$, Hardness, N.A, DS, TSS, Species*Age	21	-769.01	25.92	0.00
Species, Age, TL, $\delta^{13}C$, Hardness, N.A, DS, Species*Age	20	-761.10	33.83	0.00

Table 4.5. Species-specific attributes including species code (CHC = channel catfish, FHC = flathead catfish, NOP = northern pike, SMB = smallmouth bass, WAE = walleye), sample size (N), proportions of samples by sex (Female, Male, Unknown), mean mercury concentration and standard deviation (SD; mg/kg), maximum mercury concentration (mg/kg), mean total length (TL, mm), minimum and maximum fish length (mm), minimum, maximum and mean age, minimum, maximum and mean trophic position (TP), minimum, maximum and mean carbon signature ($\delta^{13}C$), and the number of rivers each species was sampled from. Means within a row sharing a common superscript are not significantly different (ANOVA; $P > 0.05$). R^2 and P-values refer to simple linear regressions between log-transformed mercury concentrations and total length (mm), age, trophic position, and carbon signature by species.

Attribute	Species					
Species code	CHC	FHC	NOP	SMB	WAE	All Species
N	205	123	60	176	176	740
Female (%)	51	57	57	50	40	50
Male (%)	33	41	37	49	29	37
Unknown (%)	16	2	6	1	31	13
Mean Hg	0.12 [a]	0.18 [b]	0.15 [b]	0.19 [b]	0.20 [b]	0.17
SD	0.08	0.14	0.06	0.13	0.12	0.12
Max. Hg	0.59	0.81	0.35	0.86	0.66	0.86
TL R^2/P-value	0.37/<0.01	0.62/<0.01	0.37/<0.01	0.57/<0.01	0.19/<0.01	0.14/<0.01
Age R^2/P-value	0.11/<0.01	0.65/<0.01	0.39/<0.01	0.63/<0.01	0.28/<0.01	0.07/<0.01
TP R^2/P-value	<0.01/0.51	0.29/<0.01	0.06/0.07	0.07/<0.01	0.08/<0.01	0.14/<0.01
$\delta^{13}C$ R^2/P-value	<0.01/0.71	<0.01/0.53	0.05/0.09	<0.01/0.37	0.18/<0.01	0.02/<0.01
Mean TL	460	511	579	302	401	426
Min.-Max. TL	251-721	261-1075	303-995	168-461	215-695	168-1075
Mean Age	8.3	7.5	3.9	3.1	2.8	5.3
Min.-Max. Age	1-17	1-33	1-8	1-10	0-9	0-33
Mean TP	3.03 [a]	3.59 [b]	3.64 [b,c]	3.53 [b]	3.75 [c]	3.47
Min.-Max TP	1.49-4.29	2.56-4.54	2.69-4.42	2.58-4.63	2.83-4.71	1.49-4.71
Mean $\delta^{13}C$	-24.51 [a]	-25.08 [b]	-24.77 [a,b]	-24.38 [a]	-24.57 [a]	-24.61
Min. $\delta^{13}C$	-28.23	-28.32	-27.78	-28.94	-28.76	-28.94
Max. $\delta^{13}C$	-20.76	-21.98	-22.84	-21.89	-21.17	-20.76
# of rivers	8	7	5	7	7	10

Table 4.6. Mean fish mercury concentrations (Hg; mg/kg), standard deviation (SD), and sample size (n) by species and sex. CHC = channel catfish, FHC = flathead catfish, NOP = northern pike, SMB = smallmouth bass, WAE = walleye. Means sharing a common letter within the all species category are not significantly different (ANOVA; $P > 0.05$).

Sex	Metric	CHC	FHC	NOP	SMB	WAE	All species
Female	Mean Hg	0.13	0.20	0.15	0.19	0.22	0.18 [a]
	SD	0.09	0.15	0.07	0.14	0.13	0.13
	n	104	70	34	88	71	367
Male	Mean Hg	0.11	0.15	0.16	0.19	0.22	0.17 [a]
	SD	0.07	0.13	0.06	0.12	0.12	0.11
	n	67	50	22	87	51	277
Unknown	Mean Hg	0.08	0.07	0.13	0.13	0.15	0.12 [b]
	SD	0.04	0.02	0.05	-	0.09	0.08
	n	34	3	4	1	54	96

Table 4.7. Parameter estimates (± 95% confidence intervals; C.I.) of all variables and interactions retained in the most supported fish-level and river-level models.

Variable	Parameter estimate	± 95% C.I.		Variable	Parameter estimate	± 95% C.I.	
Intercept	-0.80	-2.35	0.74	EcoregionIS	-0.31	-0.76	0.14
FHC	0.35	0.13	0.58	EcoregionLHRSP	2.29	1.19	3.39
NOP	0.50	0.19	0.81	EcoregionNILP	2.37	1.69	3.06
SMB	0.68	0.47	0.89	EcoregionPP	0.24	-0.24	0.72
WAE	0.61	0.38	0.84	EcoregionSIRLP	0.07	-0.38	0.51
SexMale	-0.16	-0.27	-0.05	Age*FHC	0.01	-0.02	0.03
SexUnk	0.04	-0.11	0.18	Age*NOP	-0.03	-0.09	0.04
Age	0.10	0.07	0.13	Age*SMB	0.07	0.04	0.11
TL	0.002	0.001	0.002	Age*WAE	0.04	-0.01	0.08
$\delta^{13}C$	0.08	0.05	0.10	SexMale*FHC	0.04	-0.13	0.21
TP	0.14	0.06	0.23	SexMale*NOP	0.28	0.07	0.50
Hardness	0.006	0.004	0.01	SexMale*SMB	0.09	-0.06	0.24
N.A	3.78	2.50	5.06	SexMale*WAE	0.35	0.18	0.53
Phos	-0.92	-1.40	-0.45	SexUnk*FHC	-0.06	-0.50	0.38
TSS	-0.001	-0.002	0.001	SexUnk*NOP	-0.26	-0.66	0.14
Sulfate	-0.02	-0.03	-0.01	SexUnk*SMB	0.04	-0.66	0.75
HTI	0.03	0.01	0.04	SexUnk*WAE	-0.02	-0.22	0.19
logperWater	-1.08	-1.44	-0.72	Age*TL	-0.0001	-0.0001	-0.00001
logperWet	0.62	0.46	0.78				
logperFor	1.49	0.96	2.01				
logperGrass	-2.32	-3.05	-1.58				

Table 4.8. Top ten multiple regression models developed to predict fish mercury concentrations using river-level variables (see Methods) ordered by Akaike's information criterion (AIC_c) using regression subset selection procedure. K = the number of parameters in the model (includes Species, Age, $\delta^{13}C$, TP, Hardness, NN, N.A, Phos, DS), ΔAIC_c = the distance of each model from the best AIC_c model, and w_i = the model weight (a measure of relative strength). Each model was produced from 740 observations.

Model	K	AIC_c	ΔAIC_c	w_i
HTI, logperWater, logperWet, logperFor, logperGrass, Ecoregion	37	-806.10	0.00	0.50
SO, HTI, logperWater, logperWet, logperFor, logperAg, logperDev, Ecoregion	38	-804.97	1.13	0.29
WA, HTI, logperWater, logperWet, logperFor, logperAg, logperDev, Ecoregion	39	-803.98	2.13	0.17
SO, WA, HTI, logperWater, logperWet, logperFor, logperAg, logperDev, Ecoregion	41	-800.04	6.07	0.03
SO, WA, HTI, logperWater, logperWet, logperFor, logperGrass, logperAg, logperDev, Ecoregion	42	-798.06	8.05	0.01
logperWater, logperWet, logperFor, logperAg, Ecoregion	35	-794.13	11.97	0.00
logperWet, logperGrass, logperAg, logperDev, Ecoregion	34	-783.34	22.76	0.00
WA, logperWet, logperFor, logperDev, Ecoregion	33	-778.39	27.71	0.00
logperWet, logperFor, logperDev, Ecoregion	32	-775.74	30.37	0.00
logperWet, logperDev, Ecoregion	31	-772.16	33.94	0.00

Table 4.9. Mean fish mercury concentrations (Hg; mg/kg), standard deviation (SD), and sample size (n) by species and ecoregion. DSML = Des Moines Lobe; IS = Iowan Surface; NILP = Northwest Iowa Loess Prairies; PP = Paleozoic Plateau; SIRLP = Southern Iowa Rolling Loess Prairies. CHC = channel catfish, FHC = flathead catfish, NOP = northern pike, SMB = smallmouth bass, WAE = walleye. Means sharing a common letter within the all species category are not significantly different (ANOVA; $P > 0.05$).

Ecoregion	Metric	CHC	FHC	NOP	SMB	WAE	All species
DSML	Mean Hg	0.13	0.17	0.14	0.14	0.14	0.15 [a]
	SD	0.10	0.15	0.05	0.03	0.15	0.12
	n	38	35	18	6	33	130
IS	Mean Hg	0.10	0.18	0.16	0.18	0.22	0.16 [a]
	SD	0.05	0.16	0.07	0.10	0.12	0.11
	n	92	34	24	93	74	317
LHSRP	Mean Hg	0.10	0.27	-	-	-	0.15[a]
	SD	0.07	0.13	-	-	-	0.12
	n	19	8	0	0	0	27
NILP	Mean Hg	0.15	-	-	0.19	0.19	0.18 [a]
	SD	0.09	-	-	0.03	0.07	0.07
	n	7	0	0	10	12	29
PP	Mean Hg	-	-	-	0.35	0.28	0.32 [b]
	SD	-	-	-	0.21	0.12	0.17
	n	0	0	0	19	18	37
SIRLP	Mean Hg	0.14	0.16	0.14	0.18	0.15	0.15 [a]
	SD	0.10	0.12	0.06	0.12	0.07	0.10
	n	49	46	18	48	39	200

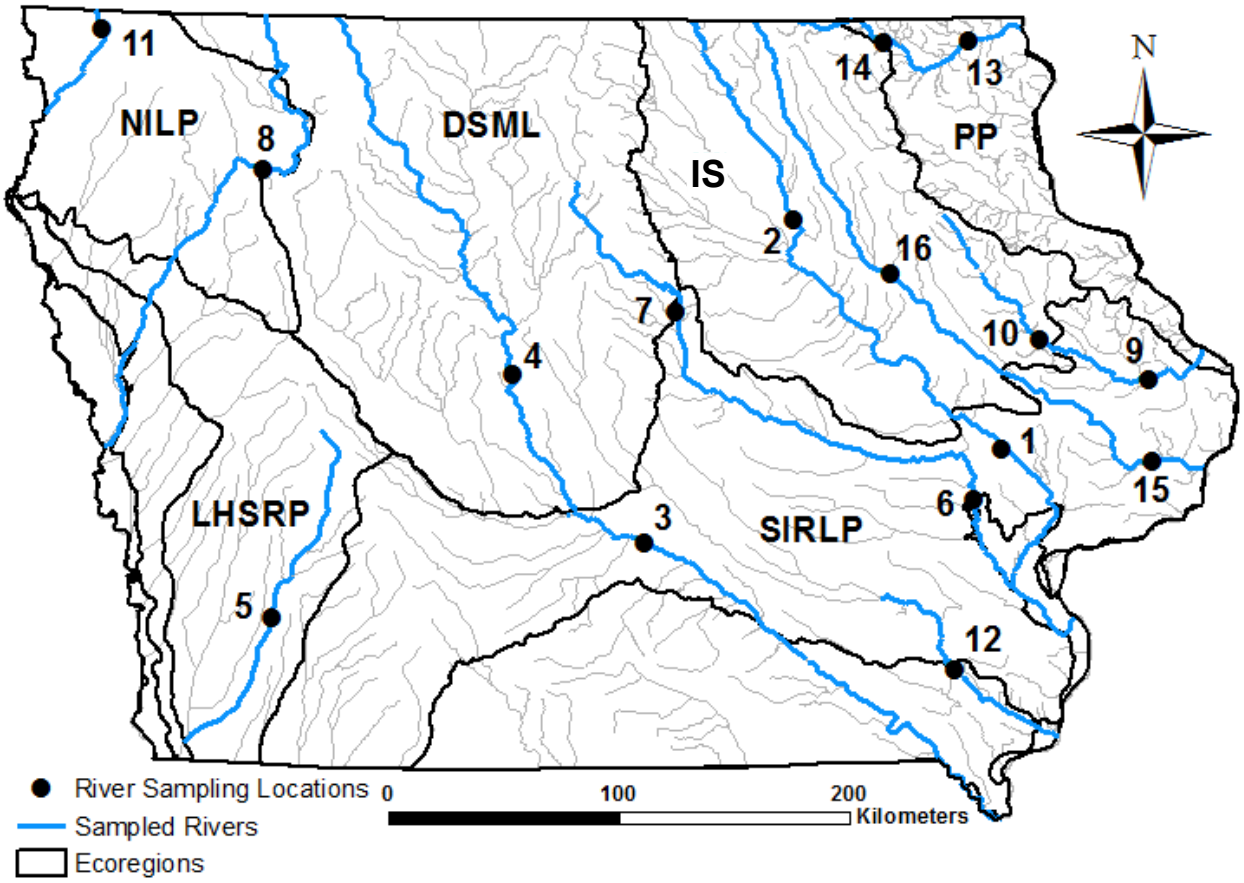


Figure 4.1. Fish sampling locations (black circles) located on 10 Iowa interior rivers. Numbers next to black dots refer to a river sampling location identification number (Table 4.1). Ecoregions: DSML = Des Moines Lobe; IS = Iowan Surface; NILP = Northwest Iowa Loess Prairies; LHSRP = Loess Hills and Steep Rolling Prairies; PP = Paleozoic Plateau; SIRLP = Southern Iowa Rolling Loess Prairies.

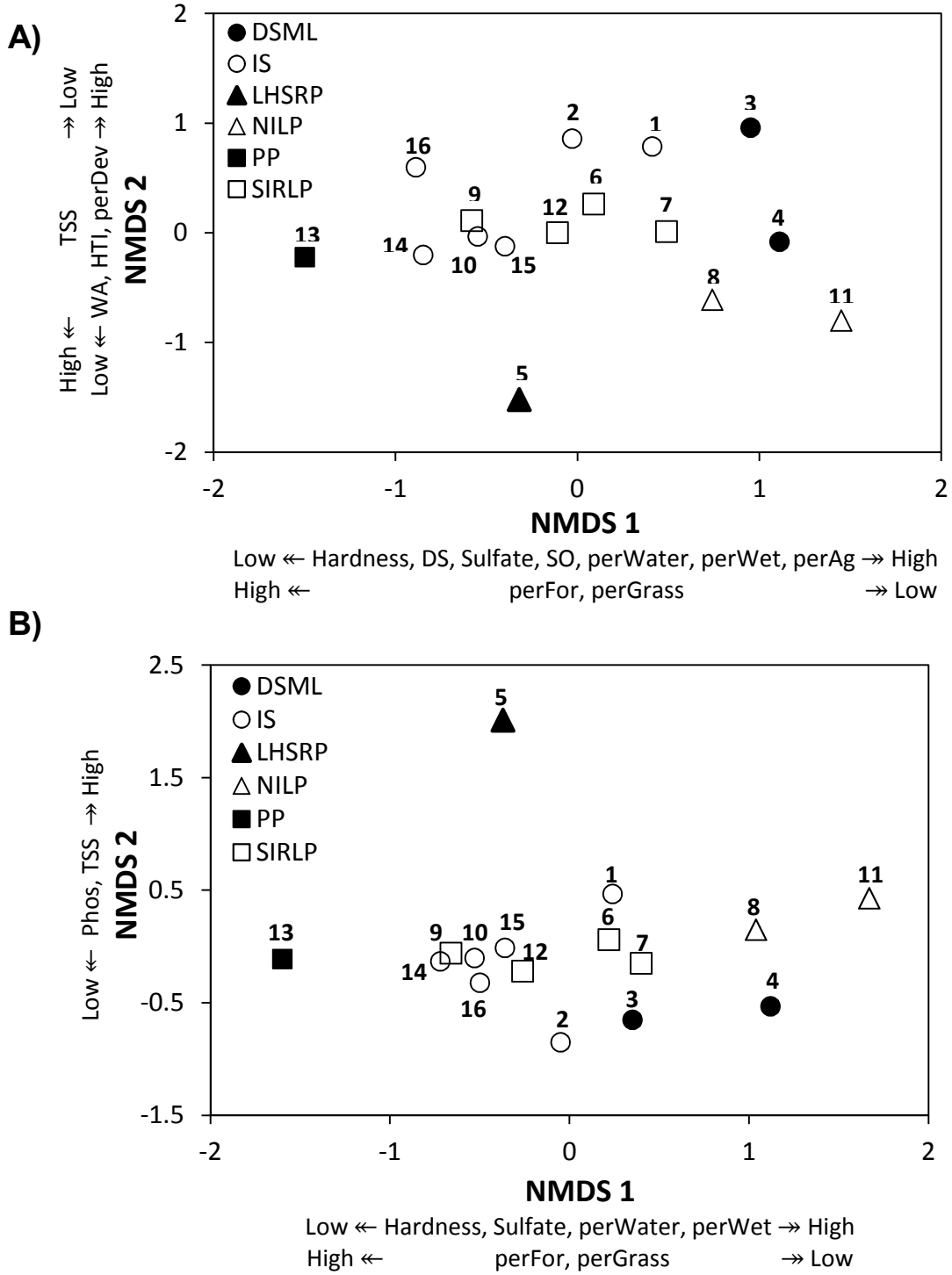


Figure 4.2. NMDS ordination of 16 reaches on 10 Iowa interior rivers based on 17 environmental variables (A), and the 10 environmental variables identified to predict fish mercury concentrations (B). River sampling reaches are coded by ecoregion. DSML = Des Moines Lobe; IS = Iowan Surface; LHSRP = Loess Hills and Steeply Rolling Prairies; NILP = Northwest Iowa Loess Prairies; PP = Paleozoic Plateau; SIRLP = Southern Iowa Rolling Loess Prairies. Identification numbers refer to river sampling location on Table 4.1. Variables significantly correlated ($r > 0.50$; $P < 0.05$) with axis scores are shown in the direction of their increase. See Appendix G for variable abbreviations.

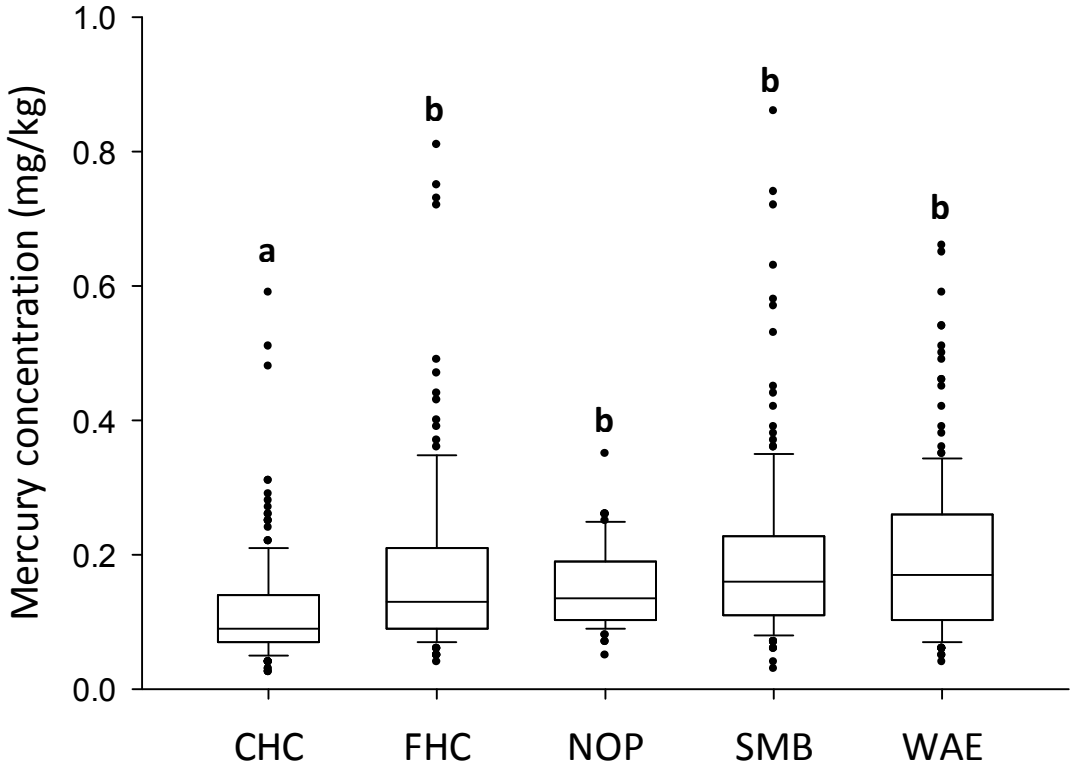


Figure 4.3. Box plots of fish mercury concentrations (mg/kg) by species. The line within the box represents the median value. Dimensions of the box represent the 25th and 75th percentiles. Error bars represent the 10th and 90th percentiles. Individual points are outliers. Boxes sharing a common letter are not significantly different (ANOVA; $P > 0.05$).

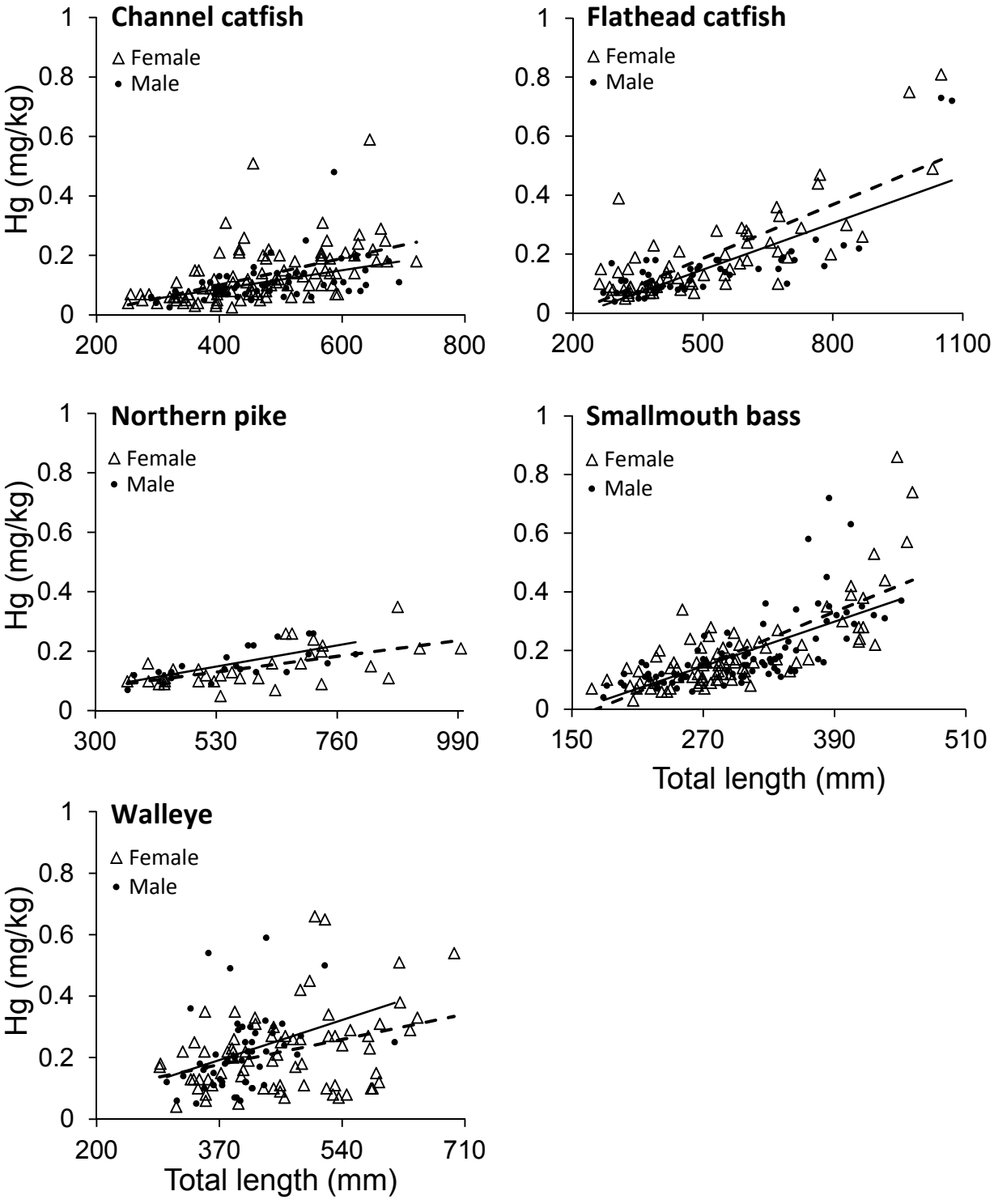


Figure 4.4. Channel catfish, flathead catfish, northern pike, smallmouth bass, and walleye mercury concentrations (mg/kg) plotted by sex (female = Δ ; male = ●) over total length (mm). Dashed line is the regression line for females; solid line is the regression line for males. See Appendix L for total length in inches.

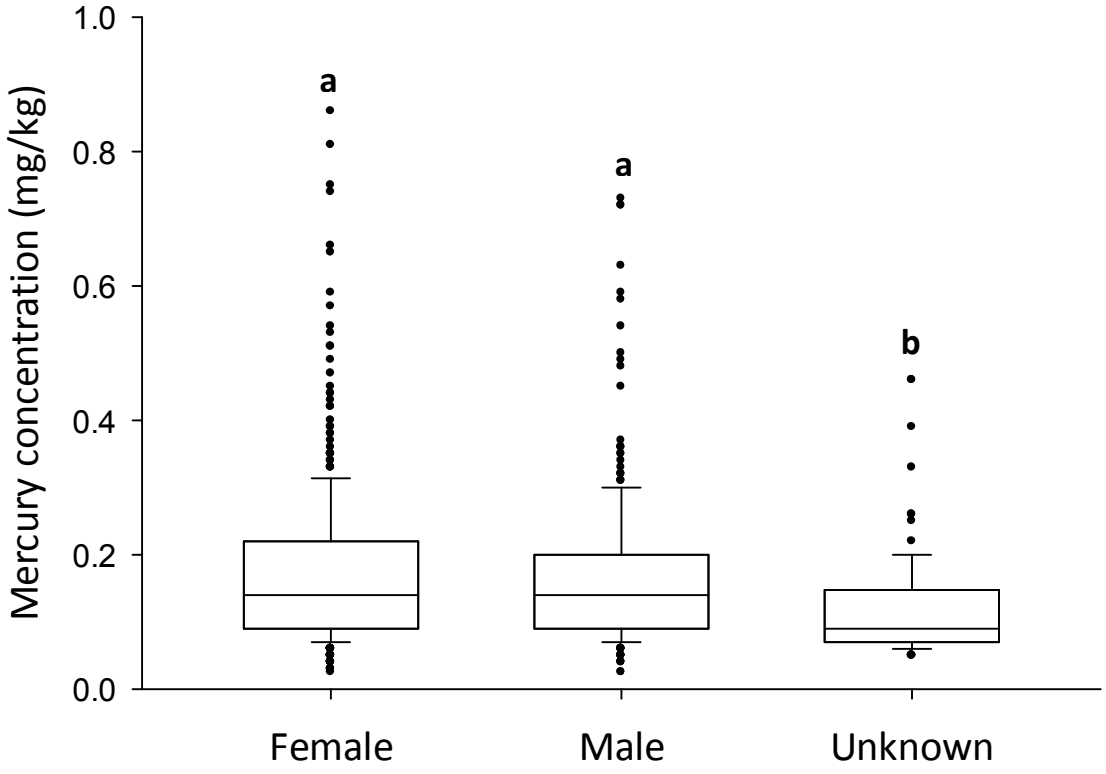


Figure 4.5. Box plots of fish mercury concentrations (mg/kg) by sex. The line within the box represents the median value. Dimensions of the box represent the 25th and 75th percentiles. Error bars represent the 10th and 90th percentiles. Individual points are outliers. Boxes sharing a common letter are not significantly different (ANOVA; $P > 0.05$).

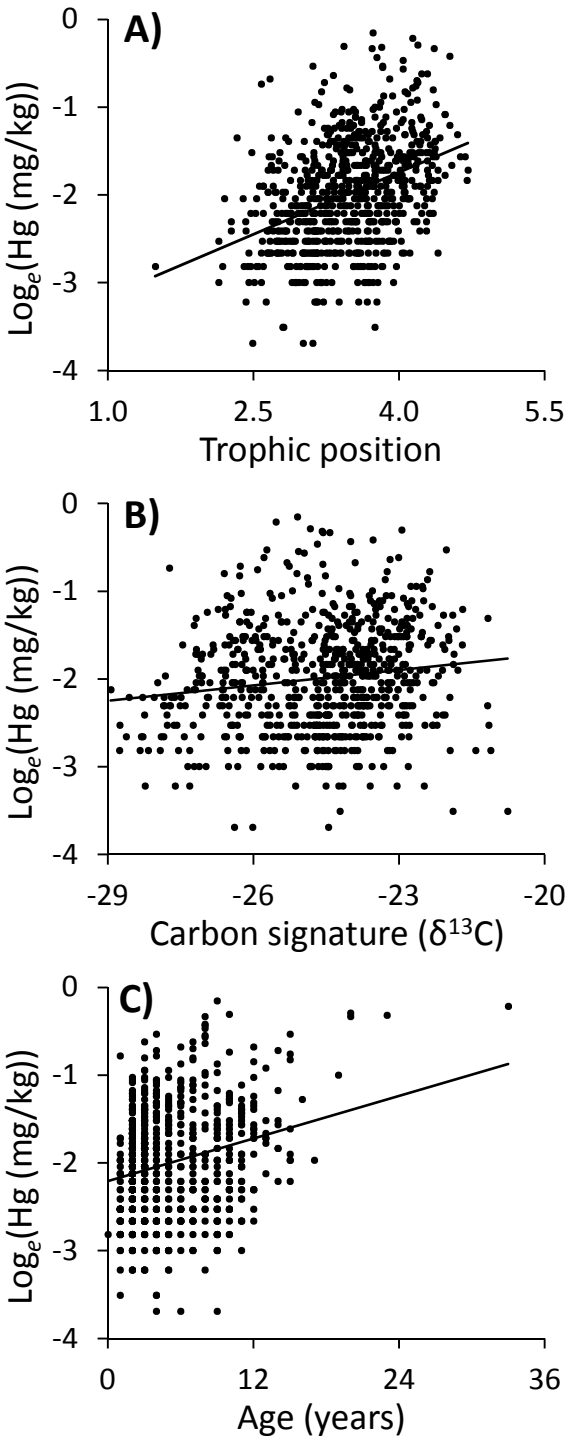


Figure 4.6. Log-transformed fish mercury concentrations (N = 740) versus trophic position (A), carbon signature (B; δ^{13}C), and age (C; years).

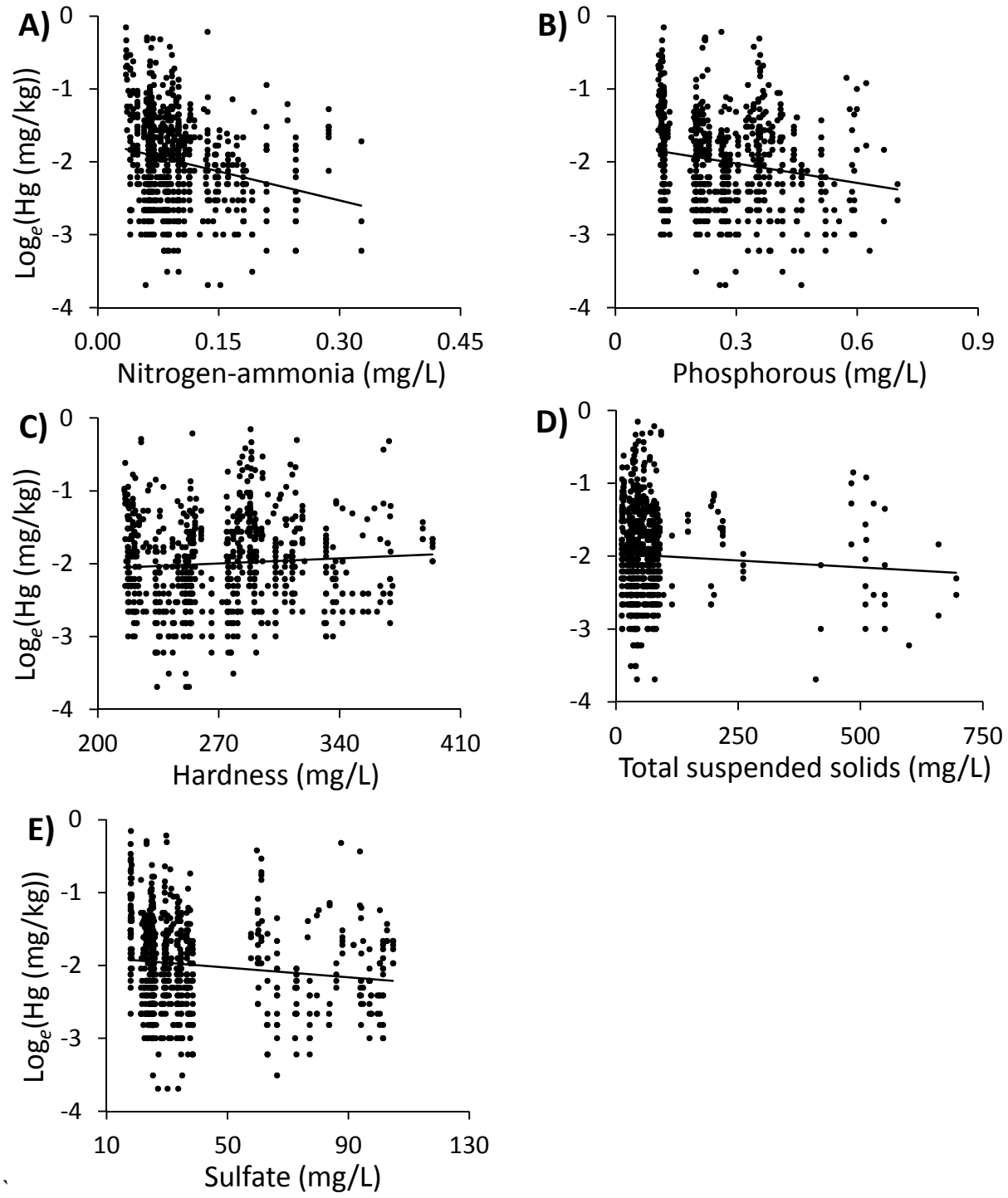


Figure 4.7. Log-transformed fish mercury concentrations (N = 740) versus nitrogen-ammonia (A; mg/L), phosphorous (B; mg/L), hardness (C; mg/L), total suspended solids (D; mg/L), and sulfate (E; mg/L).

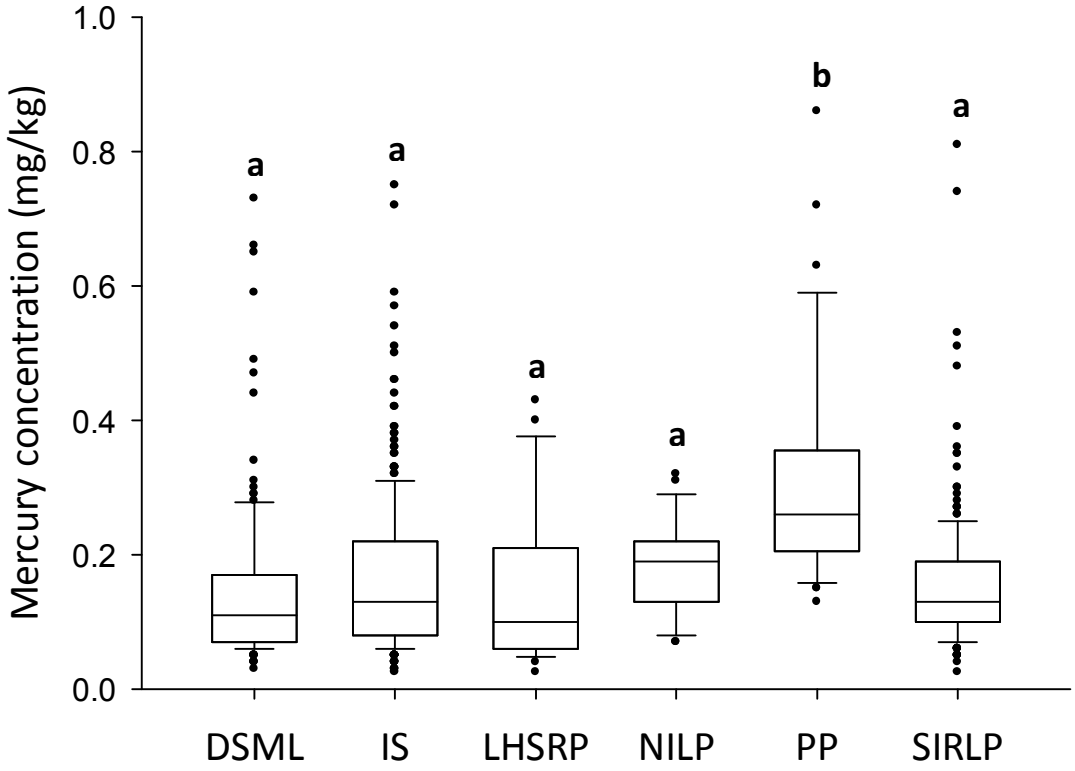


Figure 4.9. Box plots of fish mercury concentrations (mg/kg) by ecoregion. DSML = Des Moines Lobe; IS = Iowan Surface; LHSRP = Loess Hills and Steeply Rolling Prairies; NILP = Northwest Iowa Loess Prairies; PP = Paleozoic Plateau; SIRLP = Southern Iowa Rolling Loess Prairies. The line within the box represents the median value. Dimensions of the box represent the 25th and 75th percentiles. Error bars represent the 10th and 90th percentiles. Individual points are outliers. Boxes sharing a common letter are not significantly different (ANOVA; $P > 0.05$).

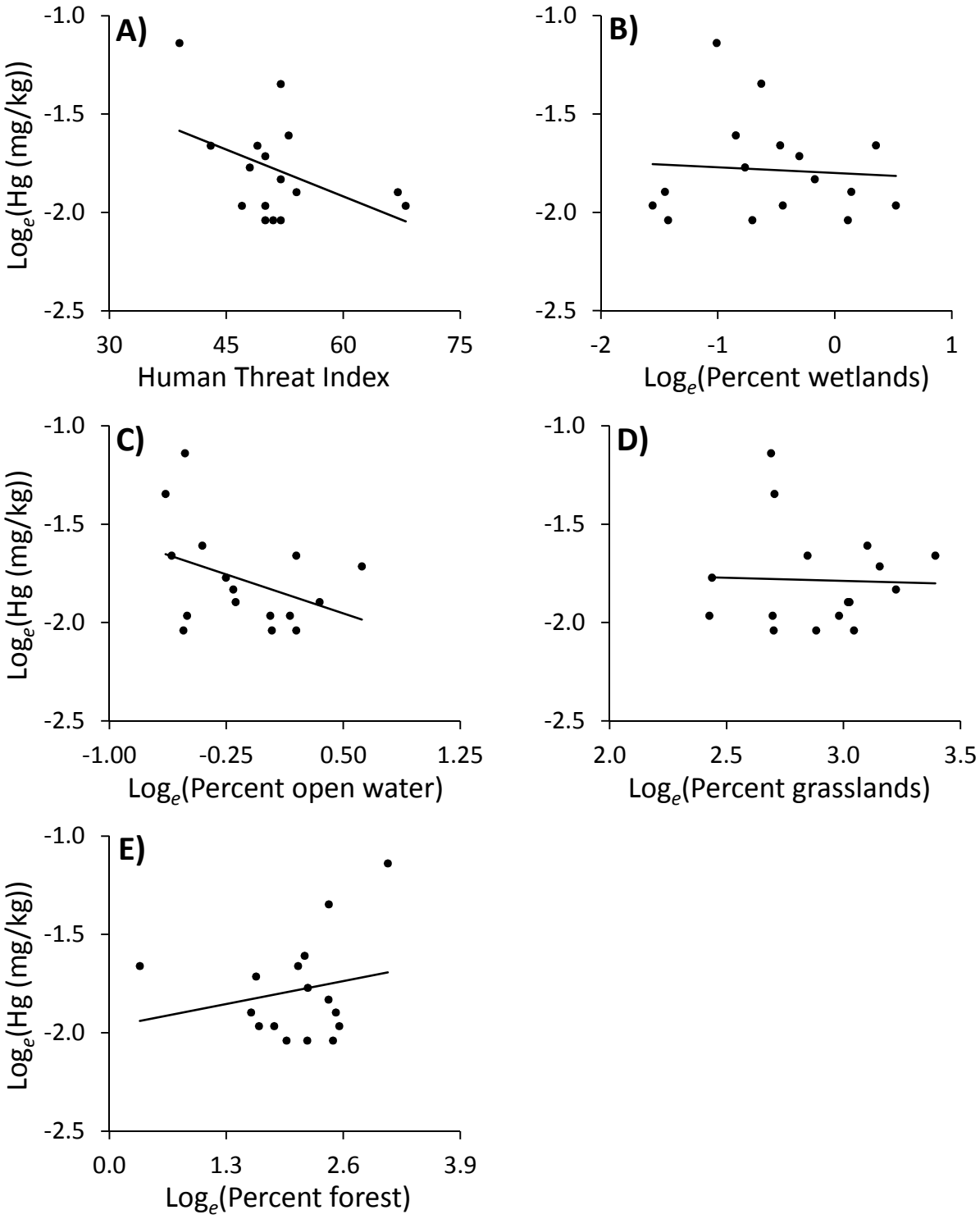


Figure 4.10. Mean log-transformed fish mercury concentrations by river (N = 16) versus log-transformed percent watershed composition variables open water (A), wetlands (B), forest (C), agriculture (D), and developed land (E).

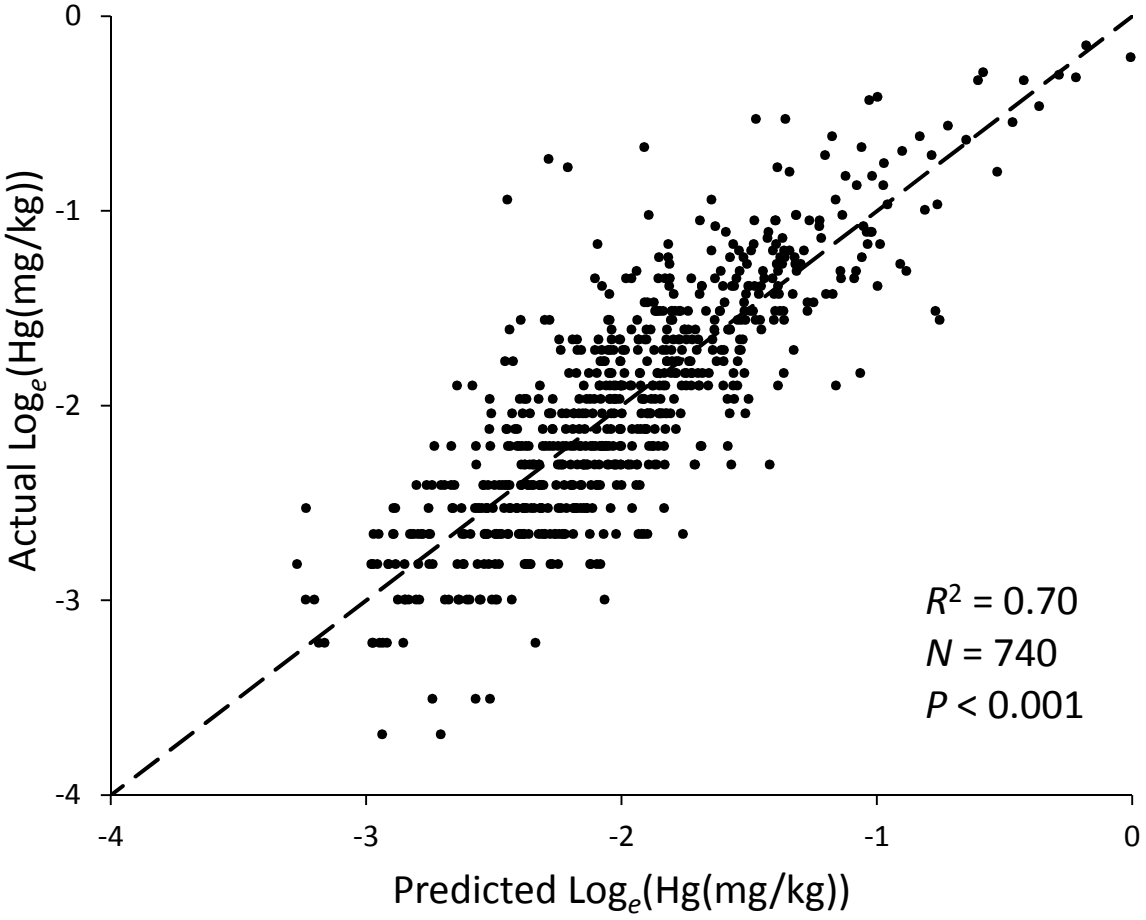


Figure 4.11. Predicted versus observed log-transformed fish mercury concentrations. The long dashed line represents the 1:1 line.

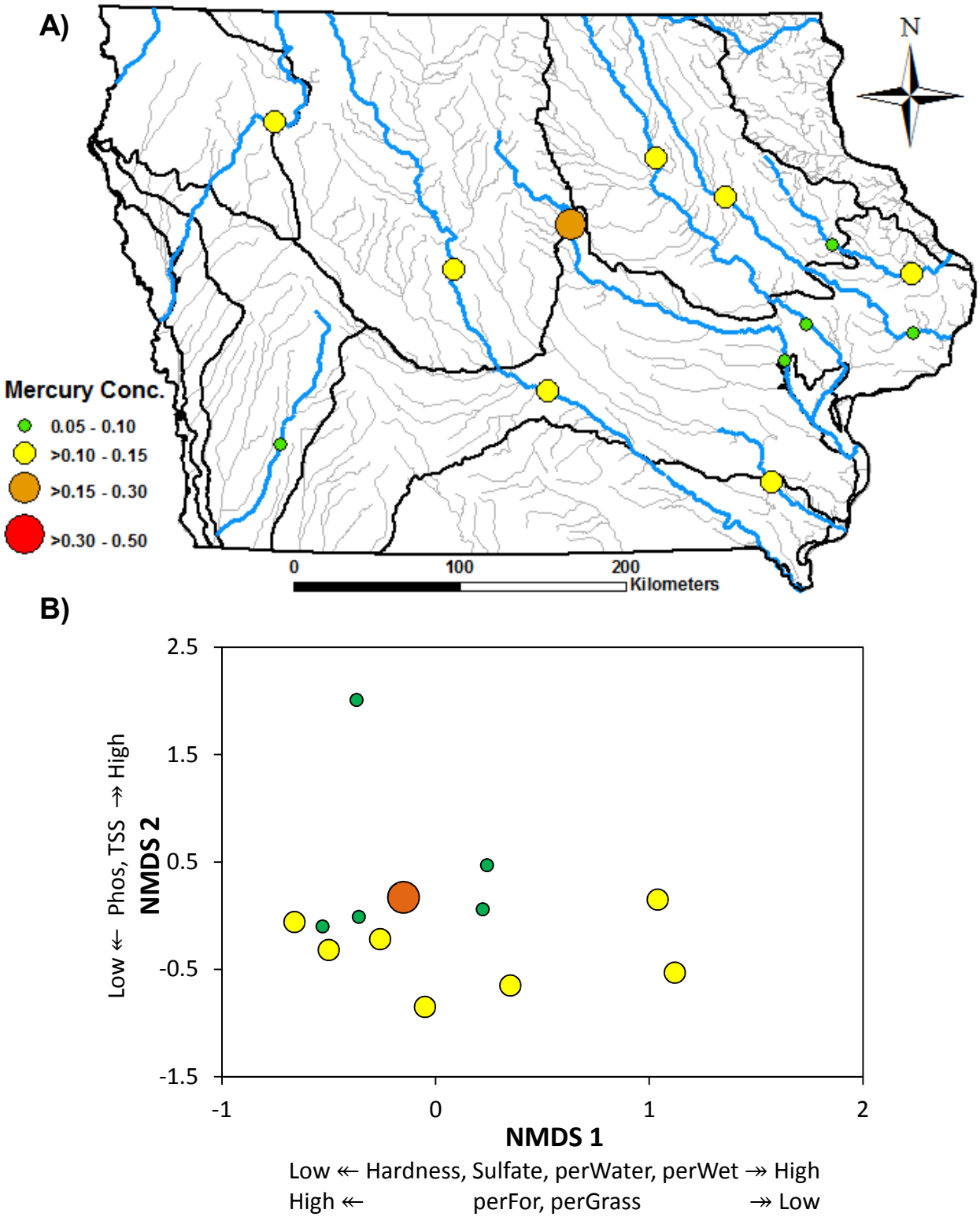


Figure 4.12. Mean channel catfish mercury concentrations by sampling location in Iowa interior rivers (A), and plotted by an NMDS ordination based on the 10 environmental variables identified as predictors of mercury concentrations (B). Only variable vectors that were significantly correlated with NMDS axes are labeled on the axes ($r \geq 0.50$, $P < 0.05$). See Appendix G or Methods for variable abbreviations.

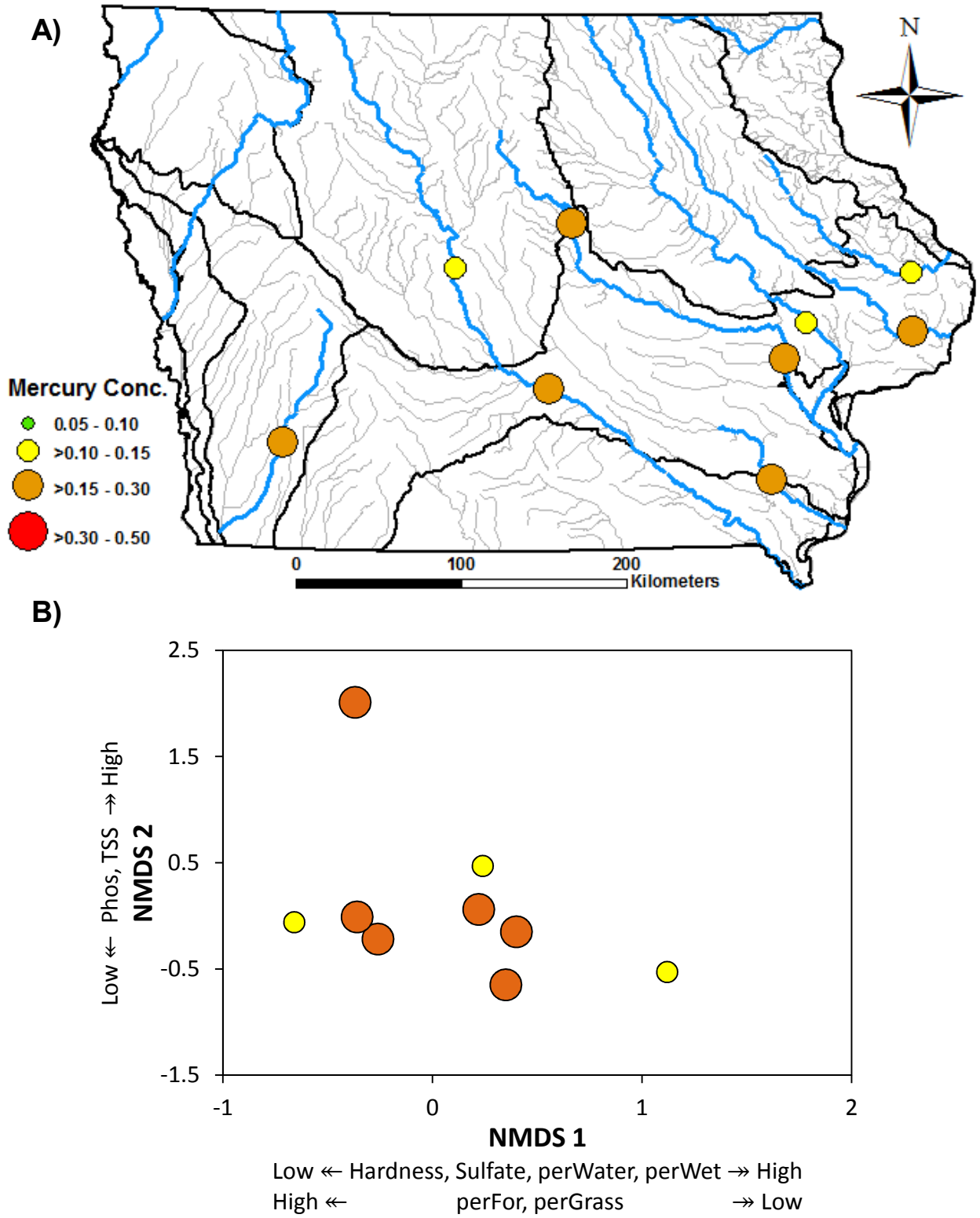


Figure 4.13. Mean flathead catfish mercury concentrations by sampling location in Iowa interior rivers (A), and plotted by an NMDS ordination based on the 10 environmental variables identified as predictors of mercury concentrations (B). Only variable vectors that were significantly correlated with NMDS axes are labeled on the axes ($r \geq 0.50$, $P < 0.05$). See Appendix G or Methods for variable abbreviations.

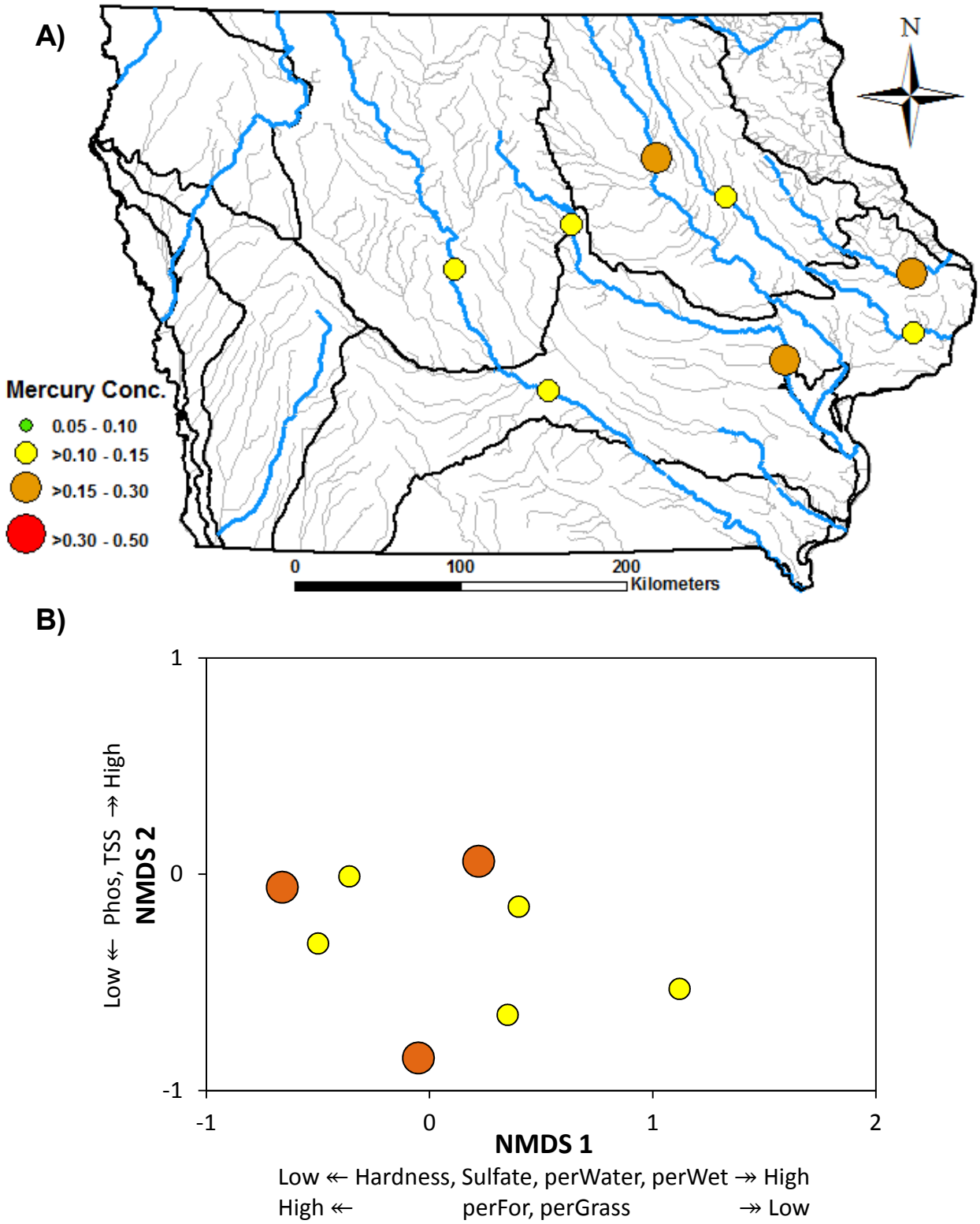


Figure 4.14. Mean northern pike mercury concentrations by sampling location in Iowa interior rivers (A), and plotted by an NMDS ordination based on the 10 environmental variables identified as predictors of mercury concentrations (B). Only variable vectors that were significantly correlated with NMDS axes are labeled on the axes ($r \geq 0.50$, $P < 0.05$). See Appendix G or Methods for variable abbreviations.

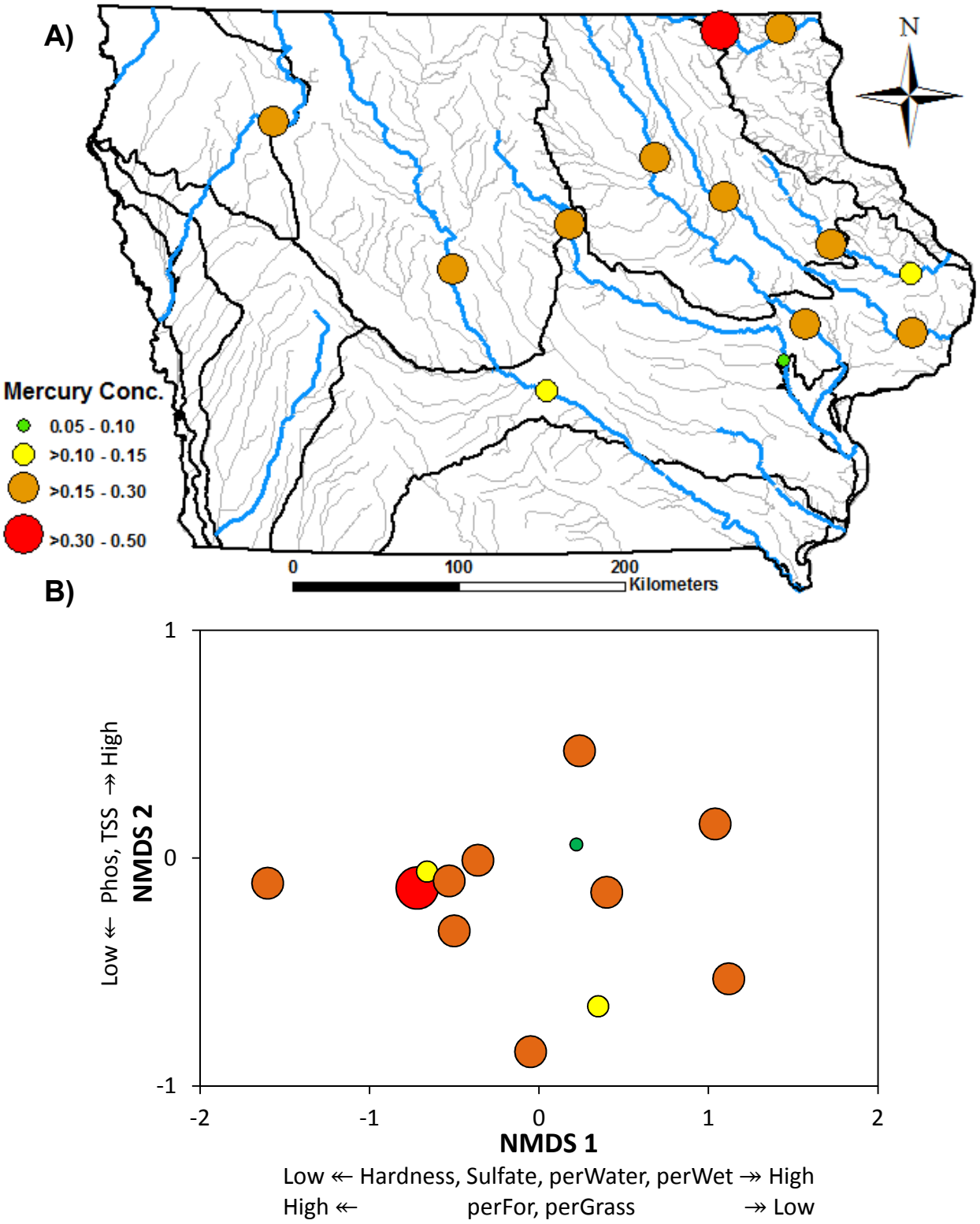


Figure 4.15. Mean walleye mercury concentrations by sampling location in Iowa interior rivers (A), and plotted by an NMDS ordination based on the 10 environmental variables identified as predictors of mercury concentrations (B). Only variable vectors that were significantly correlated with NMDS axes are labeled on the axes ($r \geq 0.50$, $P < 0.05$). See Appendix G or Methods for variable abbreviations.

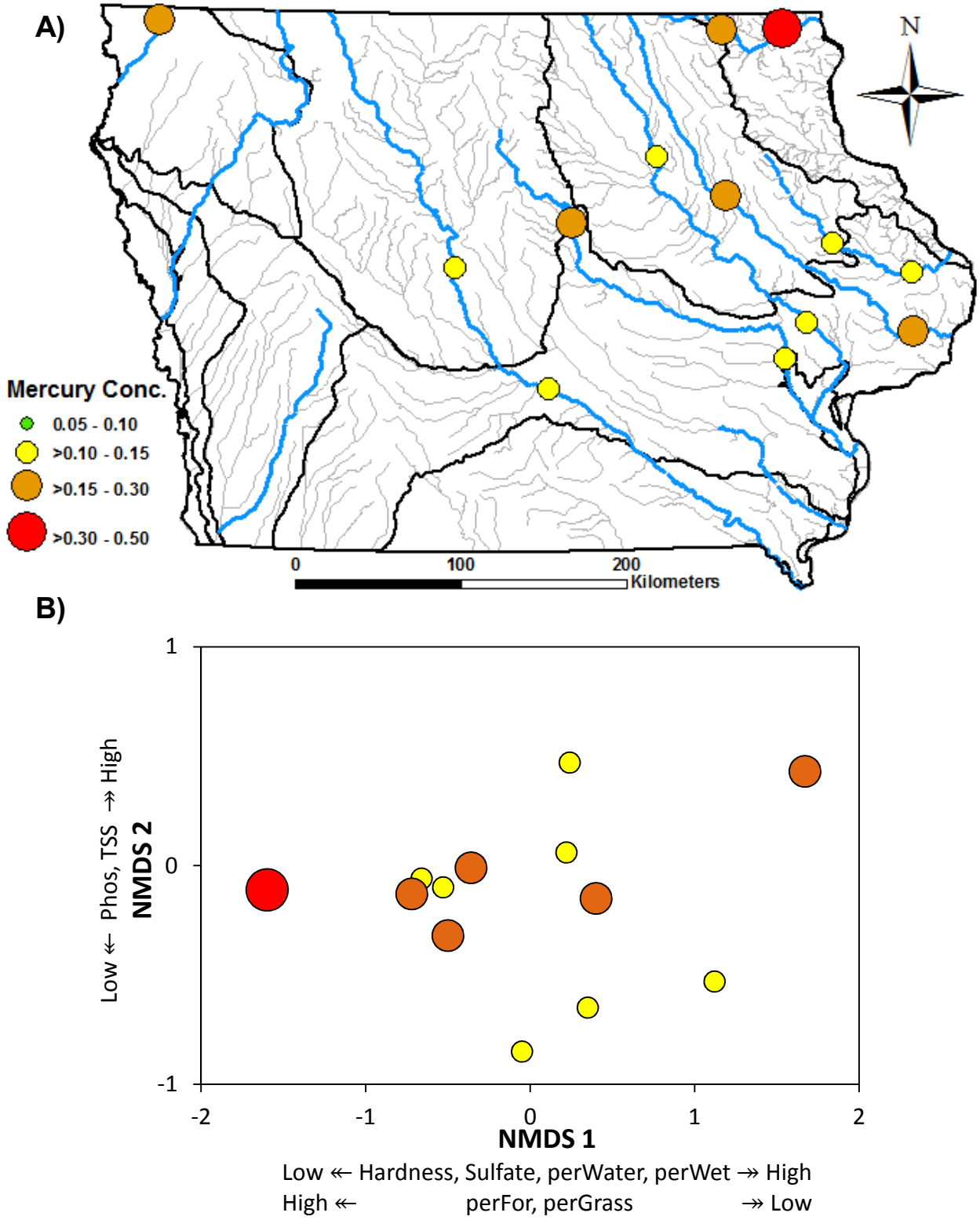


Figure 4.16. Mean smallmouth bass mercury concentrations by sampling location in Iowa interior rivers (A), and plotted by an NMDS ordination based on the 10 environmental variables identified as predictors of mercury concentrations (B). Only variable vectors that were significantly correlated with NMDS axes are labeled on the axes ($r \geq 0.50$, $P < 0.05$). See Appendix G or Methods for variable abbreviations.

CHAPTER 5

MANAGEMENT IMPLICATIONS

Currently, fish consumption advisories have been issued for relatively few Iowa lakes and rivers. However, relatively few systems have been sampled for mercury. A holistic understanding of factors influencing mercury levels in Iowa fishes is necessary to temporally standardize fish sampling protocols, predict mercury contamination in both river and lake systems that have not been sampled, and provide adequate fish consumption advisories. The goal of this study was to provide a comprehensive analysis of factors influencing mercury concentrations in Iowa fishes.

The first objective (Chapter 2) of this study provided insight to seasonal variation in largemouth bass mercury concentrations collected from two impoundments. Since 1980, the IFTMP monitoring program has sampled thousands of fishes across Iowa. However, sampling had been conducted between April-October, with little temporal guidance. Fish mercury concentrations can be higher in spring months than summer or fall months (Ward and Neumann 1999). Thus, fishes sampled in the fall could provide a conservative estimate of the maximum accumulation potential. However, seasonal variation in fish mercury concentrations was only detected in one of my two study lakes. Additionally, the maximum difference in mean mercury concentrations between months was only 0.12 mg/kg, suggesting that seasonal variation of fish mercury concentrations is rather subtle. This finding may not provide enough evidence to temporally standardize fish sampling protocols for mercury monitoring (i.e., fish may only be taken certain months of the year for mercury analysis). These findings also suggest that seasonal variation of fish mercury concentrations may occur on a lake by lake basis. Additional seasonally

stratified sampling of Iowa lakes may be necessary to identify to breadth of seasonal variation of fish mercury concentration in Iowa lakes.

The second objective of this study was to identify variables that can be used to explain and predict mercury contamination in both Iowa lakes and interior river systems. Results from this study, particularly the two predictive models, could also be used to identify potentially contaminated water bodies. There are over 800 public water bodies in Iowa and only a relatively small number have been sampled for mercury. Validation of these two models would be a crucial next step in confirming the reliability of their predictive power. The predictive models created during this project can be considered adaptive predictive models, meaning additional observations from new species and waterbodies may be added to expand the utility of the models.

In general, mercury concentrations in fishes collected from Iowa lakes and rivers are low. Altogether, this project provides necessary information to guide future mercury monitoring programs, and possibly influence fish consumption advisories throughout the state. Specifically, knowledge of mercury contamination on a statewide scale may help to simplify the existing lake by lake and river segment fish consumption advisories.

APPENDICIES

APPENDIX A. META-ANALYSIS DATA – CHAPTER 2

Summary of meta-analysis results. Reported here are authors, year of publication, common and scientific name of fish species, sample size (N), mean mercury concentrations (Hg; mg/kg), binary account of whether or not seasonal variation was detected (SV; 1 = yes, 0 = no), waterbody type, categorical trophic status, and mean total length of fishes evaluated (TL; mm; NR = not reported).

Authors	Year	Common name (*Scientific name*)	N	Mean Hg	SV (1/0)	Waterbody type	Trophic status	TL
Bae and Lim	2012	Chub mackerel (*Scomber japonicus*)	36	0.06	1	Ocean	Piscivore	337
Braaten et al.	2014	Perch (*Perca fluviatilis*)	562 (total)	0.26	1	Natural Lake	Piscivore	140
Braaten et al.	2014	Perch (*Perca fluviatilis*)	562 (total)	0.31	1	Natural Lake	Piscivore	144
Burger and Gochfeld	2011	Striped bass (*Morone saxatillis*)	178	0.39	1	Ocean	Piscivore	830
Burger and Gochfeld	2011	Bluefish (*Potamomus saltatrix*)	206	0.35	1	Ocean	Piscivore	470
Burger and Gochfeld	2011	Tautog (*Tautoga onitis*)	47	0.20	0	Ocean	Invertivore	420
Burger and Gochfeld	2011	Windowpane flounder (*Scophthalmus aquosus*)	48	0.18	0	Ocean	Omnivore	280
Burger and Gochfeld	2011	Weakfish (*Cynoscion regalis*)	60	0.15	0	Ocean	Omnivore	440
Burger and Gochfeld	2011	Northern kingfish (*Menticirrhus saxatilis*)	72	0.15	1	Ocean	Invertevore	280
Burger and Gochfeld	2011	Summer flounder (*Paralichthys dentatus*)	260	0.14	0	Ocean	Omnivore	520
Burger and Gochfeld	2011	Atlantic Croaker (*Micropogonias undulatus*)	63	0.12	0	Ocean	Omnivore	310
Burger and Gochfeld	2011	Scup (*Stenotomus chrysops*)	27	0.09	0	Ocean	Invertivore	260

APPENDIX A. Continued.

Authors	Year	Common name (*Scientific name*)	N	Mean Hg	SV (1/0)	Waterbody type	Trophic status	TL
Burger and Gochfeld	2011	Winter Flounder (*Pseudopleuronectes americanus*)	58	0.06	0	Ocean	Invertivore	NR
Burger and Gochfeld	2011	Ling (*Molva molva*)	39	0.04	1	Ocean	Omnivore	260
Costa et al.	2009	Largehead hairtail (*Trichiurus lepturus*)	104	0.13	1	Ocean	Piscivore	631
Farkas et al.	2000	Bream (*Abramis brama*)	57	0.15	0	Natural Lake	Invertivore	262
Farkas et al.	2000	Pike-perch (*Stizostedion lucioperca*)	39	0.26	0	Natural Lake	Piscivore	412
Farkas et al.	2000	Eel (*Anguilla anguilla*)	22	0.11	1	Natural Lake	Omnivore	645
Foster et al.	2000	Largemouth bass (*Micropterus salmoides*)	53	0.42	0	Reservoir	Piscivore	425
Fowlie et al.	2010	Yellow perch (*Perca flavescens*)	31	0.15	1	River	Piscivore	138
Gochfield et al.	2012	Striped bass (*Morone saxatillis*)	98	0.39	1	Ocean	Piscivore	833
Hylander et al.	2000	Pintado (*Pseudoplatystoma coruscans*)	23	0.30	1	River	Piscivore	900
Marrugo-Negrete et al.	2010	Bagre pintado (*Pseudoplatystoma fasciatum*)	24	0.43	1	Marsh	Piscivore	NR
Marrugo-Negrete et al.	2010	Mojarra (*Caquetaia kraussi*)	22	0.40	1	Marsh	Piscivore	NR
Marrugo-Negrete et al.	2010	Moncholo (*Hoplias malabaricus*)	33	0.33	1	Marsh	Piscivore	NR
Marrugo-Negrete et al.	2010	Pacora (*Plagioscion surinamensis*)	33	0.32	1	Marsh	Piscivore	NR
Marrugo-Negrete et al.	2010	Bocachico (*Prochilodos magdalenae*)	33	0.14	1	Marsh	Omnivore	NR
Marrugo-Negrete et al.	2010	Liseta (*Leporinus muyscoruma*)	27	0.26	1	Marsh	Omnivore	NR

APPENDIX A. Continued.

Authors	Year	Common name (*Scientific name*)	N	Mean Hg	SV (1/0)	Waterbody type	Trophic status	TL
Mills	2016	Largemouth bass (*Micropterus salmoides*)	129	0.18	1	Impoundment	Piscivore	318
Mills	2016	Largemouth bass (*Micropterus salmoides*)	117	0.19	0	Impoundment	Piscivore	378
Moreno et al.	2015	Northern pike (*Esox lucius*)	49	0.58	1	Natural Lake	Piscivore	514
Moreno et al.	2015	European whitefish (*Coregonus lavaretus*)	121	0.17	1	Natural Lake	Invertivore	314
Moreno et al.	2015	European perch (*Perca fluviatilis*)	96	0.42	1	Natural Lake	Piscivore	228
Murphy et al.	2007	Smallmouth bass (*Micropterus dolomieu*)	45	0.80	1	River	Piscivore	240
Özden	2010	Atlantic Bonito (*Sarda sarda*)	120	0.33	1	Ocean	Piscivore	NR
Özden	2010	Horse Mackerel (*Trachurus trachurus*)	600	0.29	1	Ocean	Piscivore	NR
Saei-Dehkordi et al.	2010	Narrow-barred Spanish mackerel (*Scomberomorus commerson*)	12	0.31	1	Ocean	Piscivore	900
Saei-Dehkordi et al.	2010	Dorah wolf-herring (*Chirocentrus dorab*)	12	0.16	0	Ocean	Piscivore	690
Saei-Dehkordi et al.	2010	Pickhandle barracuda (*Sphyraena jello*)	12	0.20	0	Ocean	Piscivore	675
Saei-Dehkordi et al.	2010	Cobia (*Rachycentron canadum*)	12	0.21	1	Ocean	Piscivore	765
Saei-Dehkordi et al.	2010	Longtail tuna (*Thunus tonggol*)	12	0.53	0	Ocean	Piscivore	565
Saei-Dehkordi et al.	2010	Largehead hairtail (*Trichiurus lepturus*)	14	0.12	0	Ocean	Piscivore	780

APPENDIX A. Continued.

Authors	Year	Common name (*Scientific name*)	N	Mean Hg	SV (1/0)	Waterbody type	Trophic status	TL
Saei-Dehkordi et al.	2010	Blacktip tevally (*Caranx sem*)	14	0.25	1	Ocean	Omnivore	440
Saei-Dehkordi et al.	2010	Silver pomfret (*Pampus argenteus*)	16	0.13	1	Ocean	Omnivore	290
Saei-Dehkordi et al.	2010	Black pomfret (*Parastromateus niger*)	16	0.18	0	Ocean	Omnivore	280
Saei-Dehkordi et al.	2010	Threadfin bream (*Nemipterus japonicus*)	10	0.18	0	Ocean	Omnivore	260
Saei-Dehkordi et al.	2010	Orange-spotted grouper (*Epinephelus coioides*)	10	0.40	1	Ocean	Piscivore	425
Saei-Dehkordi et al.	2010	Bartail flathead (*Platycephalus indicus*)	10	0.19	1	Ocean	Piscivore	375
Saei-Dehkordi et al.	2010	Indian halibut (*Psettodes erumei*)	10	0.45	1	Ocean	Piscivore	405
Saei-Dehkordi et al.	2010	Silver grunt (*Pomadasys argenteus*)	10	0.26	1	Ocean	Invertivore	490
Saei-Dehkordi et al.	2010	Yellowfin seabream (*Acanthopagrus latus*)	10	0.39	1	Ocean	Invertivore	400
Tugrul et al.	1980	Red mullet (*Mullus surmuletus*)	36	0.07	1	Ocean	Invertivore	136
Tugrul et al.	1980	Grey mullet (*Mugil auratus*)	30	0.02	1	Ocean	Invertivore	307
Ward and Neumann	1999	Largemouth bass (*Micropterus salmoides*)	75	0.66	1	Impoundment	Piscivore	329
Ward and Neumann	1999	Largemouth bass (*Micropterus salmoides*)	73	0.50	1	Impoundment	Piscivore	305

APPENDIX B. VARIABLE ABBREVIATIONS – CHAPTER 3

Variable abbreviations, descriptions, and sources for variables used in the Chapter 3 analysis.

Abbreviation	Description	Source
Species	NOP = northern pike, MUE = muskellunge, BLG = bluegill, LMB = largemouth bass, CRP = black and white crappie combined, WAE = walleye	Field
Sex	Male, female, or unknown	Lab processing
JD	Julian Day referring to sampling date	Field
Hg	Fish tissue mercury concentration (mg/kg)	Reported by State Hygienic Laboratory, Ankeny, Iowa
TL	Fish total length (mm)	Field
SW	Sample weight reported by State Hygienic Lab (mg)	Reported by State Hygienic Lab
Age	Age of fish (years)	Lab processing
Turb	Turbidity (NTU; mg/L)	Processed and reported by Iowa Lakes Information System (ILIS)
TSI	Trophic State Index – an average of chl-α, phosphorous, and Secchi depth indices.	Processed and reported by ILIS
pH	pH	Processed and reported by ILIS
Alk	Alkalinity as CaCO3 (mg/L)	Processed and reported by ILIS
SD	Secchi depth (m)	Processed and reported by ILIS
Chl-a	Chlorophyll-*a* (µg/L)	Processed and reported by ILIS
TP	Total phosphorous (µg/L)	Processed and reported by ILIS
TKN	Total Kjeldahl-nitrogen (mg/L)	Processed and reported by ILIS
VSS	Volatile suspended solids (mg/L)	Processed and reported by ILIS
TSS	Total suspended solids (g/L)	Processed and reported by ILIS
Northing	Northing coordinate (m)	Universal Transverse Mercator (UTM) Zone 15N
Easting	Easting coordinate (m)	Universal Transverse Mercator (UTM) Zone 15N
LA	Lake area (ha)	Dataset provided by the Iowa Department of Natural Resources (IDNR)
LV	Lake volume (m^2)	Dataset provided by the IDNR
MxD	Maximum depth (m)	Dataset provided by the IDNR
MD	Mean depth of lake (m)	Dataset provided by the IDNR
WA	Watershed area (km^2)	Dataset provided by the IDNR
perWater	Percent of watershed area as open water	Dataset provided by the IDNR
perWet	Percent of watershed area as wetlands	Dataset provided by the IDNR
perFor	Percent of watershed area as forest, both deciduous and coniferous	Dataset provided by the IDNR
perGrass	Percent of watershed area as grasslands	Dataset provided by the IDNR
perAg	Percent of watershed area as agricultural	Dataset provided by the IDNR
perDev	Percent of watershed area as developed	Dataset provided by the IDNR
LakeType	Categorization of lake type (SNL=Shallow natural lake, NL=Natural lake, R=Reservoir, CL=Constructed lake)	Dataset provided by the IDNR

APPENDIX B. Continued.

Abbreviation	Description	Source
Ecoregion	Level III ecoregions and sub-ecoregions: CIP = Central Irregular Plains; DSML = Des Moines Lobe; IS = Iowan Surface; LHSRP = Loess Hills and Steeply Rolling Prairies; NILP = Northwest Iowa Loess Prairies; PP = Paleozoic Plateau; SIRLP = Southern Iowa Rolling Loess Prairies	A lake is considered to be in an ecoregion if at least 50% of its watershed exists in the ecoregion. Determined using ArcGIS software.
YSC	Years since constructed lake/reservoir construction to 2015	IDNR's lake management database
WA.LA	Watershed area to lake area ratio	Calculated: watershed area (ha) divided by lake area (ha)

APPENDIX C. CORRELATION TABLE – CHAPTER 3

Correlation table between water chemistry, lake morphometric properties, and land use variables. Water chemistry variables are averaged from 2000-2015 for each lake. N = 30 for all variables except lake volume and maximum lake depth that were available for Saylorville, Red Rock, and Coralville lakes.

	Turb	pH	Alk	SD	Chl a	TP	TKN	VSS	TSS	Turb
TSI	0.79	0.06	0.10	0.14	-0.90	0.76	0.80	0.62	0.64	0.78
	0.00	0.76	0.62	0.45	0.00	0.00	0.00	0.00	0.00	0.00
Turb		0.02	0.11	0.10	-0.73	0.51	0.68	0.40	0.58	0.84
		0.92	0.56	0.59	0.00	0.00	0.00	0.03	0.00	0.00
pH			-0.22	0.09	0.15	0.40	0.06	0.58	0.35	0.16
			0.24	0.65	0.43	0.03	0.75	0.00	0.06	0.41
Alk				-0.10	-0.01	-0.22	0.30	0.01	0.10	0.21
				0.61	0.95	0.24	0.11	0.97	0.61	0.28
DOCarbon					-0.20	0.16	-0.04	0.00	0.33	0.22
					0.28	0.40	0.82	1.00	0.08	0.25
SD						-0.53	-0.58	-0.38	-0.54	-0.71
						0.00	0.00	0.04	0.00	0.00
Chl a							0.60	0.82	0.59	0.49
							0.00	0.00	0.00	0.01
TP								0.50	0.36	0.53
								0.00	0.05	0.00
TKN									0.63	0.50
									0.00	0.01
VSS										0.87
										0.00

APPENDIX C. Continued.

	Northing	Easting	MD	LV	MxD	LA	WA	WA.LA
TSI	0.35	0.11	-0.64	-0.28	-0.72	-0.06	0.10	0.14
	0.06	0.56	0.00	0.17	0.00	0.75	0.59	0.46
Turb	0.27	-0.14	-0.52	-0.13	-0.64	0.02	0.06	0.01
	0.14	0.48	0.00	0.53	0.00	0.90	0.77	0.96
pH	0.44	-0.05	-0.18	0.04	-0.34	-0.24	-0.36	-0.48
	0.01	0.79	0.34	0.84	0.09	0.20	0.05	0.01
Alk	0.45	-0.19	0.07	0.09	-0.33	0.44	0.39	0.53
	0.01	0.32	0.72	0.66	0.09	0.01	0.03	0.00
DOCarbon	-0.08	-0.31	-0.05	-0.09	-0.06	-0.12	-0.09	-0.19
	0.67	0.10	0.78	0.65	0.77	0.53	0.64	0.33
SD	-0.15	-0.07	0.57	0.27	0.60	0.01	-0.16	-0.17
	0.43	0.72	0.00	0.17	0.00	0.98	0.39	0.36
Chl a	0.38	0.07	-0.61	-0.26	-0.61	-0.37	-0.25	-0.25
	0.04	0.73	0.00	0.19	0.00	0.04	0.18	0.18
TP	0.35	0.12	-0.42	-0.21	-0.64	0.17	0.34	0.39
	0.06	0.51	0.02	0.30	0.00	0.38	0.07	0.03
TKN	0.60	0.02	-0.56	-0.21	-0.70	-0.26	-0.20	-0.21
	0.00	0.90	0.00	0.30	0.00	0.16	0.29	0.26
VSS	0.30	-0.04	-0.54	-0.14	-0.61	-0.20	-0.16	-0.23
	0.11	0.82	0.00	0.47	0.00	0.29	0.39	0.23
TSS	0.32	-0.09	-0.61	-0.15	-0.70	-0.08	-0.04	-0.08
	0.09	0.63	0.00	0.45	0.00	0.68	0.82	0.66
Northing		-0.25	-0.33	-0.07	-0.68	-0.03	-0.15	-0.12
		0.18	0.07	0.72	0.00	0.90	0.42	0.53
Easting			0.05	-0.01	0.29	0.02	0.14	0.23
			0.80	0.95	0.14	0.93	0.46	0.23
MD				0.36	0.70	0.44	0.20	0.14
				0.06	0.00	0.02	0.29	0.45
LV					0.20	0.91	0.91	-0.17
					0.31	0.00	0.00	0.41
MxD						0.06	0.34	0.12
						0.76	0.09	0.57
LA							0.76	0.59
							0.00	0.00
WA								0.85
								0.00

APPENDIX C. Continued.

	perWater	perWet	perFor	perGrass	perAg	perDev	Freq.hyp
TSI	0.22	0.10	-0.24	-0.35	0.24	0.10	-0.50
	0.24	0.59	0.20	0.06	0.21	0.59	0.00
Turb	0.37	0.21	-0.24	-0.33	0.15	0.16	-0.64
	0.04	0.27	0.21	0.08	0.43	0.40	0.00
pH	0.34	-0.05	-0.15	-0.16	0.01	-0.03	-0.12
	0.06	0.78	0.43	0.41	0.95	0.87	0.52
Alk	0.18	0.00	-0.64	-0.47	0.54	0.30	-0.55
	0.34	0.98	0.00	0.01	0.00	0.10	0.00
DOCarbon	0.31	0.00	0.09	0.18	-0.33	0.22	0.04
	0.10	0.99	0.63	0.33	0.07	0.25	0.85
SD	-0.23	-0.17	0.14	0.24	-0.10	-0.26	0.44
	0.23	0.37	0.45	0.21	0.60	0.17	0.02
Chl a	0.18	-0.05	-0.15	-0.09	0.06	-0.17	-0.18
	0.35	0.81	0.44	0.65	0.74	0.37	0.35
TP	-0.03	0.00	-0.29	-0.38	0.44	-0.06	-0.49
	0.88	0.98	0.12	0.04	0.02	0.76	0.01
TKN	0.35	0.10	-0.32	-0.34	0.22	-0.10	-0.40
	0.06	0.59	0.09	0.06	0.23	0.59	0.03
VSS	0.76	0.40	-0.24	-0.21	-0.14	0.20	-0.45
	0.00	0.03	0.20	0.27	0.46	0.28	0.01
TSS	0.63	0.28	-0.29	-0.34	0.04	0.29	-0.63
	0.00	0.13	0.13	0.07	0.85	0.12	0.00
Northing	0.37	-0.12	-0.53	-0.53	0.45	0.12	-0.55
	0.04	0.54	0.00	0.00	0.01	0.52	0.00
Easting	-0.24	0.26	0.40	-0.20	0.03	-0.15	0.26
	0.20	0.17	0.03	0.28	0.87	0.42	0.17
MD	-0.16	0.04	0.11	0.22	-0.12	0.13	0.21
	0.39	0.82	0.56	0.25	0.53	0.50	0.26
LV	0.13	0.15	-0.07	0.11	-0.10	-0.10	-0.24
	0.51	0.44	0.74	0.59	0.63	0.62	0.22
MxD	-0.45	-0.09	0.32	0.36	-0.18	-0.21	0.73
	0.02	0.64	0.11	0.07	0.37	0.31	0.00
LA	0.02	0.22	-0.17	-0.15	0.17	0.20	-0.45
	0.93	0.25	0.37	0.43	0.36	0.30	0.01
WA	-0.22	0.13	-0.09	-0.22	0.29	0.10	-0.22
	0.25	0.48	0.65	0.25	0.12	0.59	0.23
WA.LA	-0.37	0.05	-0.17	-0.34	0.49	0.02	-0.16
	0.05	0.78	0.39	0.07	0.01	0.91	0.41

APPENDIX C. Continued.

	perWet	perFor	perGrass	perAg	perDev	Freq.hyp
perWater	0.56	-0.18	-0.15	-0.34	0.44	-0.53
	0.00	0.33	0.42	0.07	0.01	0.00
perWet		0.34	-0.17	-0.37	0.09	-0.28
		0.07	0.37	0.04	0.64	0.13
perFor			0.30	-0.63	-0.21	0.46
			0.11	0.00	0.27	0.01
perGrass				-0.73	0.04	0.50
				0.00	0.85	0.01
perAg					-0.20	-0.29
					0.29	0.12
perDev						-0.32
						0.08

APPENDIX D. WATERSHED AND WATER QUALITY DATA – CHAPTER 3

Summary of watershed area (WA, km^2) and percent land use variables pertaining to each lake in the database (Chapter 3).

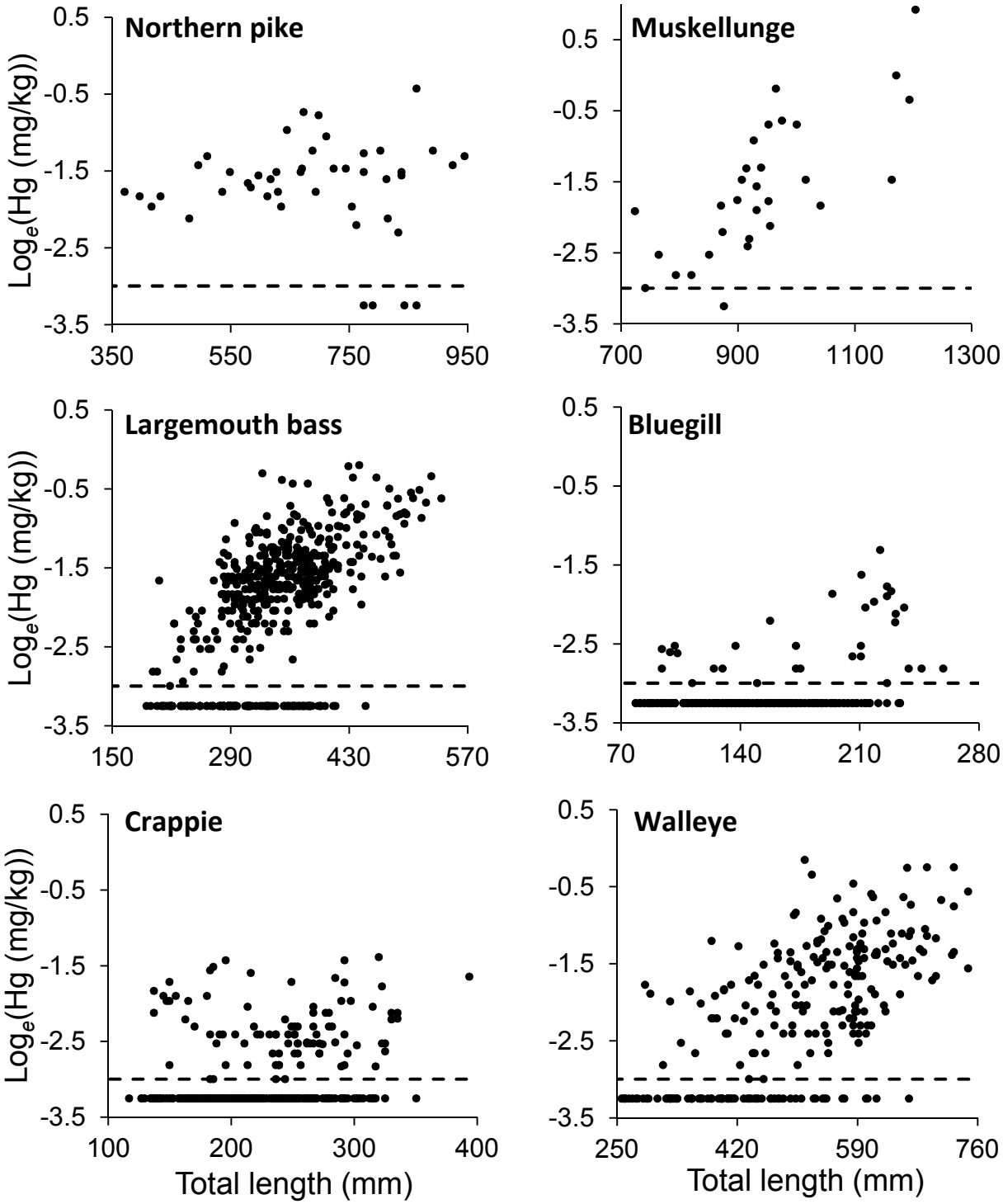
Waterbody	WA	perWater	perWet	perFor	perGrass	perAg	perDev
Ahquabi	7.5	7.8	2.0	36.3	35.5	14.4	1.2
Anita	10.1	7.0	0.2	6.1	66.1	15.0	2.4
Beeds	82.8	0.8	0.1	0.8	12.5	82.7	0.9
Big Creek	193.0	1.8	0.2	2.0	14.0	78.3	1.1
Briggs Woods	29.2	0.9	0.4	0.9	13.5	81.4	1.0
Clear	53.4	30.1	1.6	4.4	13.1	42.7	5.5
Coralville	8043.5	1.2	1.4	8.1	17.9	68.4	2.4
Crystal	9.1	12.2	0.9	1.6	25.0	59.2	0.8
Geode	41.8	1.6	0.7	16.2	19.3	58.3	1.8
Hendricks	4.9	4.1	0.4	9.8	13.6	69.3	0.8
Lake of the Hills	6.9	5.7	1.3	12.5	29.8	42.7	3.6
Little Wall	1.8	54.4	2.9	2.2	19.0	13.5	3.7
Miami	16.2	4.2	1.1	28.6	34.8	28.5	1.2
Mormon Trail	1.7	8.0	0.3	15.2	60.2	9.6	4.1
North Twin	10.3	18.4	1.4	0.5	11.9	65.4	1.3
Okoboji	77.0	22.9	1.4	5.0	32.3	31.7	5.8
Pleasant Creek	10.0	16.1	1.9	30.1	29.5	18.8	1.7
Rathbun	522.2	9.1	1.4	14.9	46.7	24.7	0.7
Red Haw	4.1	9.1	2.3	31.9	27.5	26.4	1.3
Red Rock	31916.4	1.3	1.3	8.9	17.8	67.5	2.7
Saylorville	12225.7	1.0	1.7	5.3	11.3	77.8	2.5
Silver	65.9	6.9	0.6	0.8	12.8	78.0	0.4
Spirit	91.2	27.8	1.5	2.6	17.9	48.3	0.6
Storm	72.2	18.1	0.5	1.4	12.6	60.0	5.4
Three Mile	92.0	4.4	1.1	5.9	45.2	40.2	0.9
Twelve Mile	59.3	4.9	1.0	2.5	42.2	45.6	1.1
Viking	8.8	6.8	0.2	21.4	48.8	17.4	3.3
Volga	24.6	2.4	0.7	18.8	29.2	46.5	0.9
Wapello	20.4	6.0	1.7	52.3	32.0	6.8	0.4
Yellow Smoke	6.2	3.7	0.4	4.8	44.6	41.1	1.7

APPENDIX D. Continued.

Mean water quality metrics averaged from 2000-2015. Trophic state index (TSI), turbidity (Turb, NTU), pH, alkalinity ($CaCO_3$ mg/L), dissolved organic carbon (DOC, mg/L), and frequency of hypoxic conditions (Freq.hyp).

Waterbody	TSI	Turb	pH	Alkalinity	DOC	Freq.hyp
Ahquabi	62.2	15.3	8.4	97.9	9.4	0.53
Anita	63.6	14.9	8.3	107.2	7.3	0.64
Beeds	65.2	15.4	8.1	189.0	5.3	0.55
Big Creek	55.4	8.6	8.2	175.0	4.6	0.61
Briggs Woods	58.6	7.3	8.6	145.3	6.6	0.85
Clear	63.9	37.2	8.4	145.7	9.9	0.00
Coralville	67.4	36.4	8.0	193.2	4.6	0.13
Crystal	74.6	64.3	8.7	139.1	9.7	0.15
Geode	54.6	9.7	8.7	108.9	6.4	0.72
Hendricks	66.7	15.5	8.9	82.4	7.1	0.41
Lake of the Hills	64.5	12.2	8.3	146.3	5.7	0.55
Little Wall	71.5	51.2	8.6	170.4	19.6	0.00
Miami	70.2	59.6	8.1	91.1	8.1	0.33
Mormon Trail	54.2	6.3	8.4	108.9	6.5	0.80
North Twin	66.4	36.5	8.4	148.7	9.7	0.00
Okoboji	50.0	10.7	8.4	188.6	7.1	0.03
Pleasant Creek	54.8	8.8	8.4	124.4	7.4	0.90
Rathbun	54.8	9.1	8.4	125.1	5.9	0.34
Red Haw	56.8	19.9	8.2	87.6	7.8	0.76
Red Rock	63.0	25.6	8.1	184.7	7.7	0.18
Saylorville	63.2	26.3	8.1	196.2	6.3	0.25
Silver	65.6	41.9	8.5	160.6	9.3	0.00
Spirit	55.1	17.7	8.5	170.3	10.1	0.03
Storm	66.1	57.7	8.4	158.3	7.5	0.00
Three Mile	57.3	9.1	8.1	112.2	7.3	0.80
Twelve Mile	60.7	40.7	8.3	103.2	7.3	0.73
Viking	61	15.0	8.3	115.3	41.2	0.85
Volga	64.3	17.7	8.3	127.4	6.5	0.79
Wapello	56.4	14.5	8.3	82.8	7.6	0.70
Yellow Smoke	49.5	4.3	8.4	184.1	6.2	0.50

APPENDIX E. FISH LENGTH VERSUS MERCURY RELATIONSHIPS – CHAPTER 3



Log-transformed northern pike (n = 45), muskellunge (n = 30), largemouth bass (n = 502), bluegill (n = 275), crappie (n = 315), and walleye (n = 248) mercury concentrations (mg/kg) over total length (mm). Dashed line represents the detection limit (-2.99).

APPENDIX F. MISSOURI AND MISSISSIPPI RIVER DATA – CHAPTER 3

Mean fish mercury concentrations (Hg; mg/kg), standard deviation (SD), and sample size (n) by species from fishes collected from the Missouri River and Upper Mississippi River (pools 11 and 13). NOP = northern pike, MUE = muskellunge, BLG = bluegill, LMB = largemouth bass, CRP = black and white crappie combined, WAE = walleye.

River	Metric	CHC	FHC	NOP	SMB	WAE	All Species
Missouri	Mean Hg	0.19	0.17	-	-	-	0.18
	SD	0.12	0.12	-	-	-	0.12
	n	11	13	0	0	0	24
UMR Pool 11	Mean Hg	-	-	0.28	0.22	-	0.26
	SD	-	-	0.14	0.15	-	0.15
	n	0	0	20	13	0	33
UMR Pool 13	Mean Hg	0.08	0.19	-	-	0.17	0.16
	SD	0.02	0.15	-	-	0.12	0.13
	n	15	23	0	0	18	56

APPENDIX G. VARIABLE ABBREVIATIONS – CHAPTER 4

Variable abbreviations, descriptions, and sources for variables used in the Chapter 4 analysis.

Abbreviation	Description	Source
Species	CHC = channel catfish, FHC = flathead catfish, NOP = northern pike, SMB = smallmouth bass, WAE = walleye	Field
Sex	Male, female, or unknown	Lab processing
JD	Julian Day referring to sampling date	Field
Hg	Fish tissue wet-weight mercury concentration (mg/kg)	Reported by State Hygienic Laboratory, Ankeny, Iowa
TL	Fish total length (mm)	Field
Age	Age of fish (years)	Fish processing in lab
TP	Trophic position	Calculated based on $\delta^{15}N$ signature
$\delta^{13}C$	Carbon signature	Reported by ISU Stable Isotope Laboratory
Hardness	As carbonate ($CaCO_3$; mg/L)	Processed and reported by IDNR 2015
NN	Nitrate-nitrite (mg/L)	Processed and reported by IDNR 2015
N.A	Nitrogen-ammonia (mg/L)	Processed and reported by IDNR 2015
pH	pH	Processed and reported by IDNR 2015
Phos	Phosphate-phosphorous (mg/L)	Processed and reported by IDNR 2015
DS	Dissolved solids (mg/L)	Processed and reported by IDNR 2015
TSS	Total suspended solids (mg/L)	Processed and reported by IDNR 2015
Sulfate	Sulfate (mg/L)	Processed and reported by IDNR 2015
Ecoregion	Level III ecoregions and sub-ecoregions: DSML = Des Moines Lobe; IS = Iowan Surface; NILP = Northwest Iowa Loess Prairies; PP = Paleozoic Plateau; SIRLP = Southern Iowa Rolling Loess Prairies	Ecoregions are assigned to river sampling location based on which ecoregion makes up the majority of the watershed area. This was determined using ArcGIS software.
SO	Stream order	Part of the HTI database developed by Annis et al. 2010.
WA	Watershed area (km^2)	Determined using ArcGIS software.
HTI	Human Threat Index	Part of the HTI database developed by Annis et al. 2010.
perWater	Percent of watershed area as open water	Part of the HTI database developed by Annis et al. 2010.
perWet	Percent of watershed area as wetlands	Part of the HTI database developed by Annis et al. 2010.
perFor	Percent of watershed area as forest, both deciduous and coniferous	Part of the HTI database developed by Annis et al. 2010.
perGrass	Percent of watershed area as grasslands	Part of the HTI database developed by Annis et al. 2010.
perAg	Percent of watershed area as row crop agriculture	Part of the HTI database developed by Annis et al. 2010.
perDev	Percent of watershed area as developed	Part of the HTI database developed by Annis et al. 2010.

APPENDIX H. CORRELATION TABLE – CHAPTER 4

Pearson correlation table among water chemistry and land use variables used in the Chapter 4 analysis. Water chemistry variables are averaged from 2000-2015 for each river. N = 14 for all correlations. Top number is r-value and bottom number *P*-value.

	NN	N.A	Ortho	pH	Phos	DS	TSS	TVSS	Sulfate
Hardness	0.11	-0.07	0.24	0.48	0.41	0.91	0.49	0.51	0.76
	0.70	0.82	0.41	0.08	0.14	0.00	0.08	0.06	0.00
NN		0.03	0.19	-0.08	0.06	-0.02	-0.30	-0.12	-0.14
		0.91	0.52	0.78	0.84	0.94	0.30	0.68	0.64
N.A			0.39	-0.09	0.48	0.14	0.01	0.17	0.20
			0.16	0.76	0.08	0.63	0.96	0.56	0.51
Ortho				0.29	0.86	0.37	-0.04	-0.06	0.27
				0.32	0.00	0.19	0.90	0.85	0.35
pH					0.43	0.47	0.24	0.33	0.30
					0.13	0.09	0.40	0.25	0.29
Phos						0.56	0.41	0.44	0.50
						0.04	0.15	0.12	0.07
DS							0.42	0.56	0.93
							0.14	0.04	0.00
TSS								0.86	0.42
								0.00	0.14
TVSS									0.62
									0.02

APPENDIX H. Continued.

	SO	Local HTI	Watershed HTI	Overall HTI	WA
Hardness	0.28	0.12	-0.37	-0.14	0.09
	0.32	0.69	0.20	0.64	0.76
NN	0.05	-0.23	0.19	-0.02	-0.25
	0.87	0.43	0.51	0.94	0.40
N.A	0.39	-0.10	0.21	0.05	0.49
	0.17	0.72	0.47	0.87	0.08
Ortho	0.67	0.21	0.35	0.37	0.76
	0.01	0.47	0.22	0.20	0.00
pH	0.34	0.12	0.11	0.17	0.24
	0.24	0.69	0.71	0.56	0.41
Phos	0.68	0.10	0.23	0.21	0.68
	0.01	0.73	0.44	0.48	0.01
DS	0.53	0.21	-0.11	0.08	0.40
	0.05	0.48	0.71	0.80	0.16
TSS	0.06	-0.06	-0.39	-0.30	-0.10
	0.84	0.84	0.17	0.31	0.74
TVSS	0.27	-0.15	-0.10	-0.18	0.00
	0.36	0.61	0.73	0.55	0.99
Sulfate	0.55	0.28	0.00	0.19	0.48
	0.04	0.33	0.99	0.53	0.09
SO		0.15	0.60	0.46	0.77
		0.62	0.02	0.10	0.00
Local HTI			0.23	0.81	0.40
			0.42	0.00	0.16
Watershed HTI				0.76	0.48
				0.00	0.09
Overall HTI					0.56
					0.04

APPENDIX H. Continued.

	perWater	perWet	perFor	perGrass	perAg	perBarren	perDev
Hardness	0.46	0.56	-0.16	-0.01	0.05	-0.02	-0.30
	0.10	0.04	0.58	0.97	0.87	0.96	0.29
NN	-0.04	0.03	-0.25	-0.30	0.30	-0.33	0.04
	0.89	0.91	0.39	0.31	0.30	0.24	0.90
N.A	0.38	0.14	-0.32	-0.27	0.28	-0.22	0.50
	0.18	0.64	0.27	0.35	0.33	0.45	0.07
Ortho	0.53	0.60	-0.10	-0.05	0.02	0.05	0.29
	0.05	0.02	0.72	0.86	0.95	0.87	0.31
pH	0.31	0.38	-0.09	-0.01	0.01	0.15	0.28
	0.29	0.18	0.75	0.97	0.98	0.61	0.33
Phos	0.77	0.58	-0.31	-0.19	0.19	0.02	0.12
	0.00	0.03	0.29	0.53	0.52	0.94	0.69
DS	0.64	0.72	-0.37	-0.27	0.28	-0.12	-0.08
	0.01	0.00	0.19	0.35	0.34	0.69	0.79
TSS	0.54	0.04	-0.13	0.11	0.00	0.15	-0.60
	0.05	0.90	0.65	0.71	0.99	0.61	0.02
TVSS	0.62	0.18	-0.50	-0.34	0.42	-0.07	-0.36
	0.02	0.53	0.07	0.23	0.14	0.81	0.21
Sulfate	0.69	0.64	-0.49	-0.45	0.44	-0.24	-0.07
	0.01	0.01	0.08	0.10	0.11	0.41	0.81
Stream Order	0.69	0.52	-0.29	-0.29	0.24	0.14	0.36
	0.01	0.06	0.32	0.31	0.42	0.64	0.21
Local HTI	0.33	0.14	-0.21	-0.06	0.12	-0.36	0.19
	0.25	0.62	0.46	0.84	0.68	0.20	0.51
Watershed HTI	0.32	0.19	-0.46	-0.54	0.50	-0.23	0.61
	0.27	0.51	0.10	0.05	0.07	0.43	0.02
Overall HTI	0.40	0.22	-0.41	-0.36	0.37	-0.38	0.51
	0.15	0.45	0.15	0.20	0.19	0.18	0.06
WA	0.51	0.49	-0.15	-0.21	0.13	0.04	0.48
	0.06	0.07	0.61	0.47	0.66	0.89	0.08
perWater		0.54	-0.55	-0.38	0.42	-0.27	0.06
		0.05	0.04	0.18	0.14	0.35	0.83
perWet			-0.42	-0.37	0.35	-0.23	0.16
			0.14	0.19	0.23	0.43	0.58
perFor				0.87	-0.96	0.73	-0.21
				0.00	0.00	0.00	0.48

APPENDIX H. Continued.

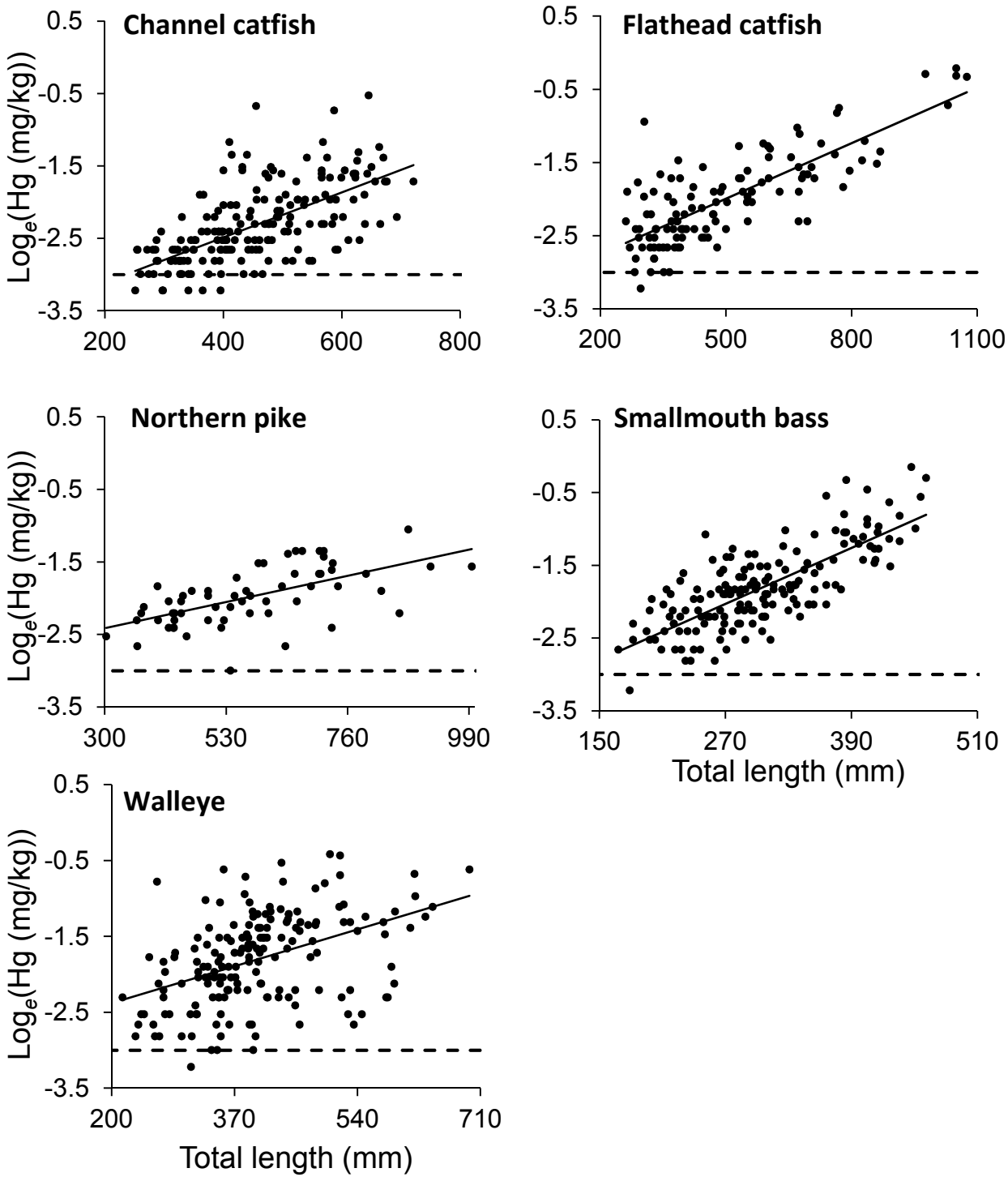
	perAg	perBarren	perDev
perGrass	-0.97	0.73	-0.30
	0.00	0.00	0.30
perAg		-0.77	0.24
		0.00	0.42
perBarren			-0.25
			0.40

APPENDIX I. WATER QUALITY DATA – CHAPTER 4

Summary table of water quality information for each river sampling location. Analytes included are hardness ($CaCO_3$ mg/L), nitrate + nitrite (NN; mg/L), nitrogen (ammonia; N.A; mg/L), pH, phosphate-phosphorous (phos; mg/L), dissolved solids (DS; mg/L), total suspended solids (TSS; mg/L), and sulfate (mg/L). Water quality metrics are averaged from 2000-2015. U = upstream sampling location, D = downstream sampling location.

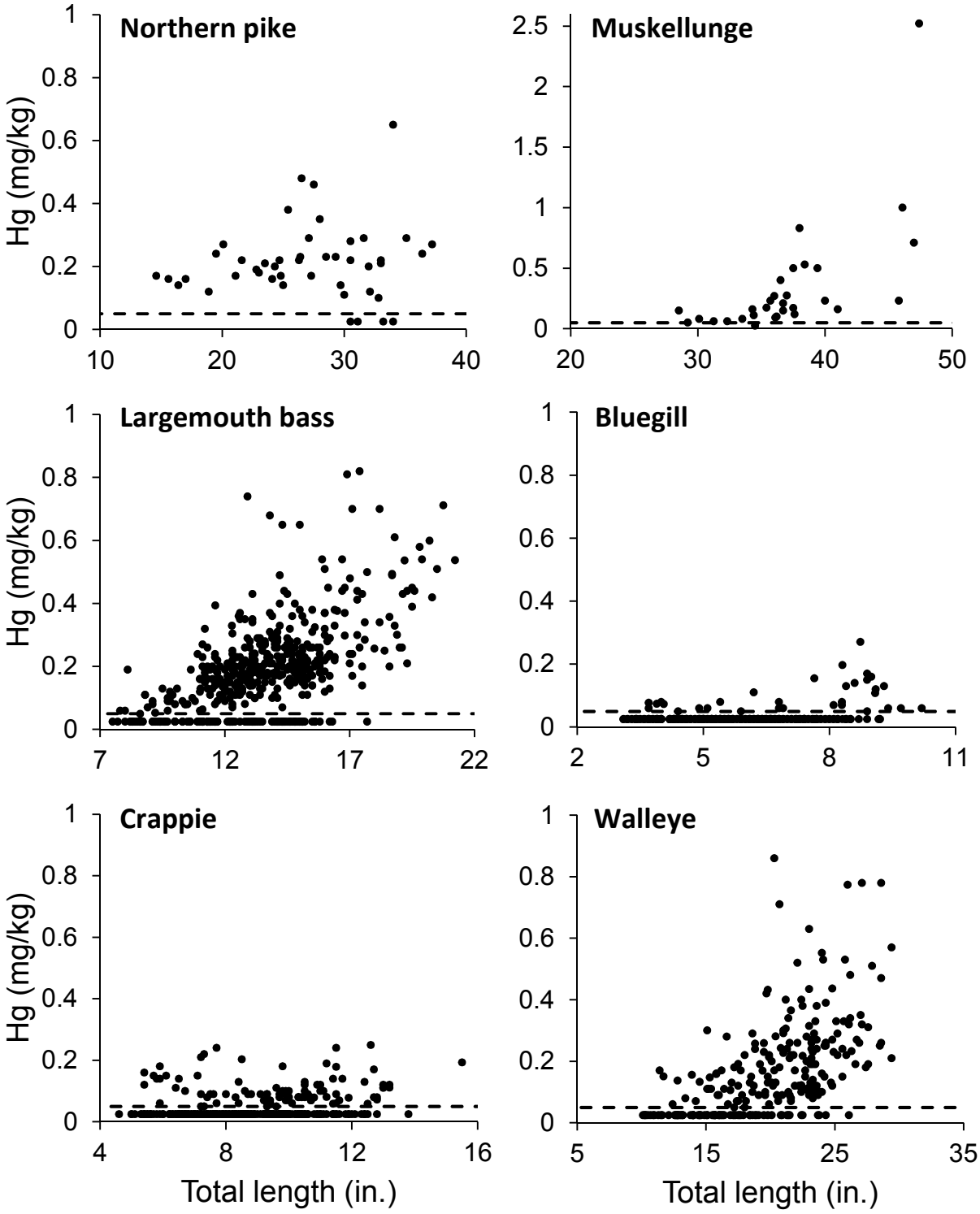
River (U/D)	Hardness	NN	N.A	pH	Phos	DS	TSS	Sulfate
Cedar (D)	252.9	5.8	0.18	8.3	0.30	329.8	46.7	35.6
Cedar (U)	255.1	6.3	0.05	8.3	0.17	303.6	32.4	25.9
Des Moines (D)	295.3	4.9	0.09	8.2	0.36	392.4	56.4	61.1
Des Moines (U)	368.4	5.9	0.08	8.3	0.22	481.9	53.1	87.4
Iowa (D)	254.6	5.0	0.14	8.1	0.26	318.6	77.9	29.6
Iowa (U)	319.6	7.6	0.07	8.3	0.36	370.7	67.0	31.1
Little Sioux	359.5	5.2	0.09	8.2	0.32	445.8	181.9	79.4
Maquoketa (D)	282.8	6.6	0.07	8.2	0.19	311.5	62.0	24.3
Maquoketa (U)	282.8	6.6	0.07	8.2	0.19	311.5	62.0	24.3
Skunk River	275.1	4.1	0.04	8.3	0.23	348.3	77.6	37.5
Upper Iowa (D)	288.5	5.0	0.05	8.2	0.16	309.7	69.5	18.4
Upper Iowa (U)	288.5	5.0	0.05	8.2	0.16	309.7	69.5	18.4
Wapsipinicon (D)	225.0	5.2	0.06	8.2	0.22	264.7	91.7	23.1
Wapsipinicon (U)	218.2	6.0	0.06	7.9	0.12	265.5	17.2	24.8

APPENDIX J. FISH LENGTH VERSUS MERCURY RELATIONSHIPS – CHAPTER 4



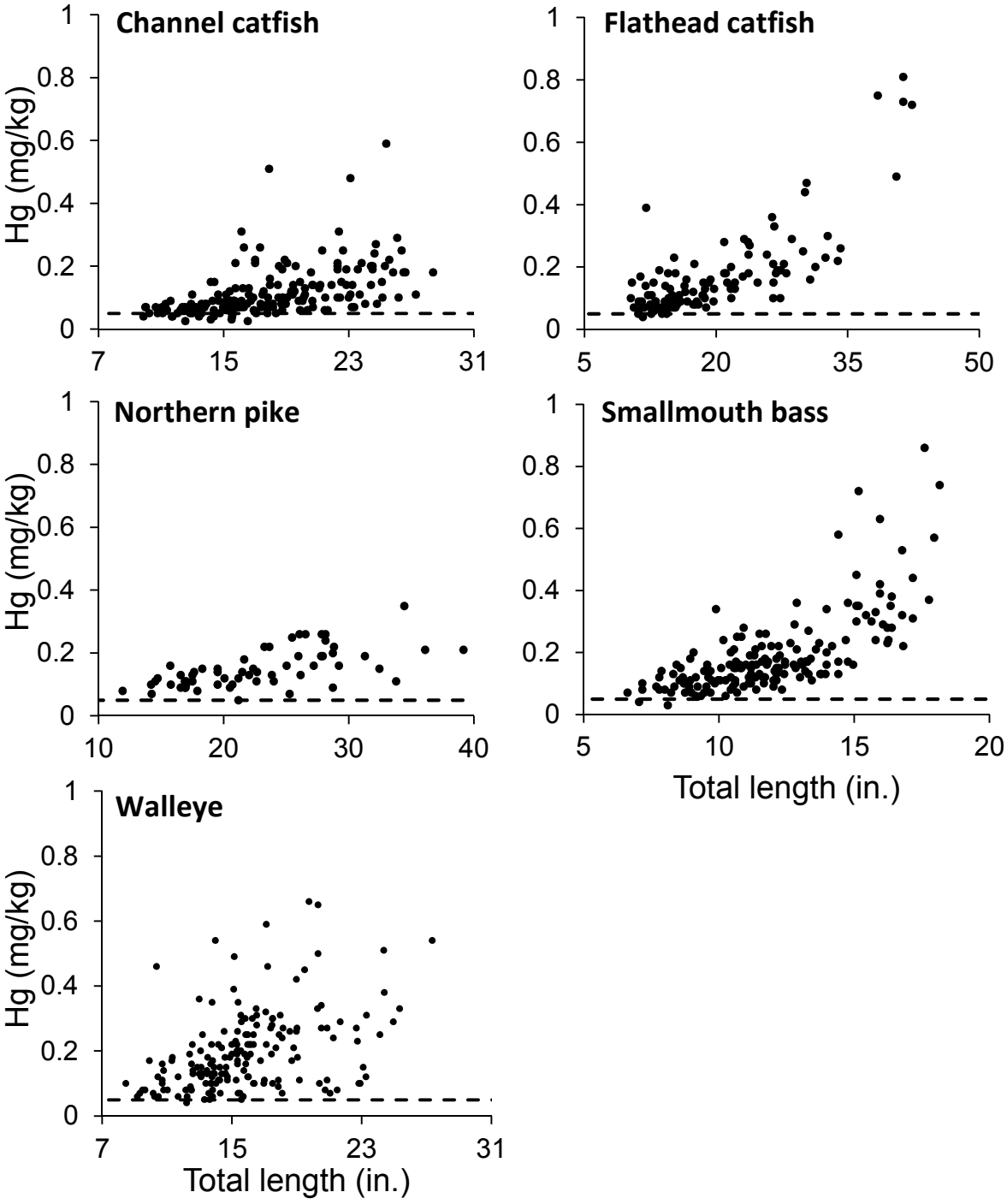
Channel catfish, flathead catfish, northern pike, smallmouth bass, and walleye log-transformed mercury concentrations (mg/kg) plotted over total length (mm). Solid line represent a simple linear regression line between total length and log-transformed mercury concentrations. Dashed line represents the log-transformed detection limit (-2.99).

APPENDIX K. FISH LENGTH IN INCHES VERSUS MERCURY– CHAPTER 3



Northern pike, muskellunge, largemouth bass, bluegill, crappie, and walleye mercury concentrations (Hg; mg/kg) versus total length (in.). Dashed line represents the detection limit (0.05). Note: muskellunge have a different y-axis scale compared to the other five species.

APPENDIX L. FISH LENGTH IN INCHES VERSUS MERCURY– CHAPTER 4



Channel catfish, flathead catfish, northern pike, smallmouth bass, and walleye mercury concentrations (Hg; mg/kg) versus total length (in.). Dashed line represents the detection limit (0.05).